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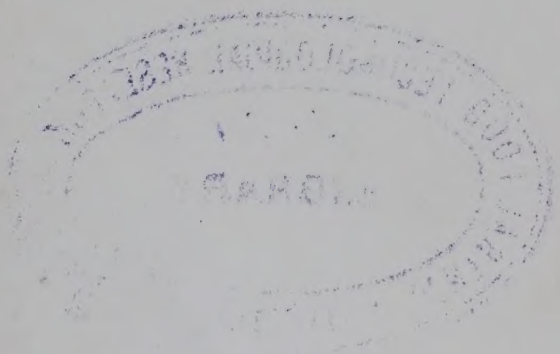
# CALCIUM METABOLISM



# CALCIUM METABOLISM

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# Contents

# CHAPTER I

# Introduction

CALCIUM is the most important inorganic element in the body, nd occurs in it in the highest amount. It is somewhat sur-rising that in human diets, at any rate, the supply is so xiguous. The highest Ca content of foods is of course in bone, nd it is a regular agricultural practice to feed farm animals n bone-meal. This dietary practice has never appealed to ivilized man, who has to rely on dairy products and certain reen vegetables for his supply of Ca. In spite of this small istribution in the diet, Ca deficiency is not common, and the eason for this will be explained in more detail later.

The form in which Ca occurs in foods has not been much nvestigated. In milk it is probably associated in part with the rotein fractions, and in vegetables it must in part be in a imple soluble form since some of it is easily leached out by ater during cooking.

In the intestine an acid reaction is necessary for the solution nd absorption of Ca. Thus $Ca_3(PO_4)_2$, insoluble at neutral eaction, is readily available for utilization in the body. How-ver, in human studies there is little description of Ca defi-iency due to achlorhydria, though this has been caused experi-nentally. The degree of absorption of Ca is difficult to etermine, owing to the concurrent processes of secretion of igestive juices containing Ca, and the possible active excretion f Ca in addition. Taking the total balance of intake and out-ut into account, one finds a very low figure of retention in umans, but a considerably higher one in animals like rats.

The absorption of Ca is governed by a number of factors, ne chief of which is vitamin D. It seems probable that the

degree of absorption is also controlled by the needs of th moment, especially during the growing period, though this ha not yet been proved.

After absorption, Ca can exist in at least three forms in th body—Ca ions in solution, Ca bound to organic compounds and Ca present in a crystal lattice as in bone. A close equi librium exists between these three forms, governed by th endocrine glands and the dietary status, and obeying the rule of physical chemistry as closely as most biological reactions

The excretion of Ca is controlled by factors still incom pletely understood. The faecal Ca in man consists largely o unabsorbed dietary Ca, together with an unknown amount o secreted and actively excreted Ca. The kidney excretes C continually, even during starvation, but this may be due t the fact that the kidney tubule can deal with only certain type of Ca compounds. The urinary production of Ca is controlle by several endocrine glands.

The time-honoured method of investigating Ca metabolism is by the metabolic balance experiment. This involves a accurate knowledge of Ca intake and excretion and is tediou and often unsatisfactory to carry out. Errors can easily creep in and the full co-operation of the subject is essential. Whe errors do occur, they usually cause a fictitiously high positive balance of the element under consideration. As a result many writers have condemned balance methods, but unfortunately they have substituted nothing better in their place. The more recent use of radioactive isotopes has given us another too which can be used freely in animal experiments, but which has naturally been sparingly used with human subjects.

Tetany was first described by Steinheim over 100 years ago and Gley [1] in 1893 recognized the role of the parathyroids as regulators of Ca metabolism. In 1918 Osborne and Mende [2], working with rats, found that while the body could adapt to deprivation of most mineral elements, it was unable to adapt, either by substituting other elements or conserving what it had, to absolute Ca deficiency. The question of dietary Ca requirements was first put on a quantitative basis by Sherman

1920 [3], and while his premisses were not in all cases correct, he started an aspect of the problem which is not yet solved. H. H. Mitchell and his school have thoroughly probed the question of utilization and retention of Ca and the effects of the body stores upon these [4].

The role of Ca in bone structure has been investigated with access by biologists working in collaboration with crystallographers, especially by Hodge and his colleagues and by Ballemagne. During the last two decades our knowledge of the physiology and biochemistry of bone formation has greatly advanced; the metabolic changes in calcification appear to follow the carbohydrate phosphorylytic cycle found in other parts of the body [5]. Much remains to be sorted out, especially the function of phosphatase. Bone formation has also been studied from the point of view of both endocrine and dietary influences [6, 7].

The teeth, until recently the Cinderellas of the calcified tissues, are now receiving their due share of attention, largely in an attempt to solve the caries problem. Apart from this, much fundamental work has appeared from Schour and his colleagues in Chicago, from the Bethesda group, and from other workers such as Bevelander, Armstrong and Sognnaes. These aspects will be expanded in the following pages. And further it will be seen that Ca, although an apparently inert mineral element, undergoes some surprising adventures between its advent in the mouth and its final excretion.

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## CHAPTER II

# Content and Availability of Calcium in Foods

THE Ca content of common foodstuffs has now been widely etermined and many tables giving the composition of foods ave been published. The best source of Ca in human diets is ilk or milk products excluding butter. Cows' milk contains bout 0·12 per cent. of Ca and goats' milk has slightly more. kimmed milk, liquid or dried, is just as good a source of Ca s whole milk. Cheddar cheese contains about 800 mg. Ca per 00 g. and thus is a very good source of Ca, but acid types of ottage cheese have only about 1/9th of the Ca content of heddar cheese. Human milk contains far less Ca than cows' ilk. Leitch [1] has surveyed the literature on this and reported gures ranging from 0·013 to 0·04 per cent., the Ca content eing to some extent modified by the diet of the mother. It is n interesting coincidence, as pointed out by Bunge [2], that ne Ca content of the milk has a relationship with the rate of rowth of the young, as these figures show:

| *Species* | *Days to double birth weight* | *Ca % in mother's milk* |
|---|---|---|
| Man | 180 | 0·02 |
| Cow | 47 | 0·12 |
| Dog | 7 | 0·32 |

The other main dietary source of Ca is green vegetables, nd especially the outer green leaves, which may contain as uch as twenty to thirty times as much Ca as the inner

ones [3]. The methods of growing vegetables can influen their composition, artificial fertilizers tending to lower the and raise the K content [4], the Ca possibly being replaced some other element.

McCance and his colleagues [5] have analysed a number vegetable foodstuffs. The most interesting conclusions a those relating to the effects of methods of preparation a cooking on the final Ca content of the food. They investigat the effects of steaming, boiling, baking, frying and roasting the common constituents of vegetables. The only procedu affecting Ca is boiling. Boiling scarlet runner beans for minutes and carrots for 120 minutes caused a loss of betwe 12 and 20 per cent. of the Ca in the cooking water. This w much less than the loss of, for example, Cl and K, of whi over 60 per cent. were dissolved in the case of beans. Additi of alkali to the water made no difference to the loss. A mo recent American study [6] showed that cooking cabbage water resulted in a loss of over 20 per cent. of Ca by leachi but with pressure cooking only 9 per cent. was lost. Averagi the results from eleven vegetables investigated, the same w found, nearly 25 per cent. of Ca being lost if the vegetable w covered with cooking water, and much less when the wat was considerably reduced. It must be admitted that the content of many of these vegetables is so low that these loss make little difference to the daily dietary intake. The out leaves of cabbage contain 0·429 per cent., but carrots ha only 0·045 per cent. and beans 0·055 per cent.

Several foods now have Ca added to them, the commone being bread. In England, chalk is added to the flour to ove come the action of phytic acid, and in the U.S.A. dried mi and improvers are added, considerably increasing the content. Prouty and Cathcart [7] found that the Ca content fresh bread in the U.S. might vary from 0·065 per cent. 0·141 per cent., six slices of bread giving 30 per cent. of t daily requirement, supposing all the Ca to be available.

One can summarize by saying that the bulk of our Ca inta comes from milk or milk products, taken either as such or

arious cooked foods. The Ca from vegetable sources provides nly a small fraction of the total daily intake.

*Availability of Ca in foodstuffs.* A knowledge of this is more nportant biologically than that of the Ca content in total nount, since it may be that the Ca in the given foodstuff is ot in a condition to be nutritionally useful. This is especially ue in the case of spinach and other chenopodiaceae, the Ca which is in the form of oxalate and quite unavailable for e in the body. Spinach contains so much oxalate that it may so precipitate and render useless Ca in other constituents of e diet. In fact, Sherman [8] says that 'spinach was a mistaken oice for popularization as a typical green-leaf vegetable'. It ll take some time before the feats of the seaman associated th this vegetable are forgotten.

The dipyridyl method can be used for testing the availability iron, but no such procedure is available for Ca and rather dious metabolic balance experiments have to be employed. any experiments have been done on animals, especially lves and rats. Milk Ca is taken as the reference standard d the availability of other forms of Ca compared with it. most all inorganic forms of Ca seem to be available to some gree, such as carbonate, sulphate, phosphate and even icate [9]. Ca lactate is also a good form of Ca and supported biological functions in rats, except growth, as effectively as e Ca of milk [10].

As already stated, the Ca of spinach is not available, and anching removed very little of it, the Ca being quite insoluble d not extractable. The Ca of cauliflower and broccoli is not available as that of milk [11], nor is that of carrots, fresh tuce or beans. Cooking of carrots or beans did not alter the ailability [12].

These animal experiments have given useful information, t owing to species differences cannot necessarily be directly nsferred to man. In general it has been found that Ca from rganic sources is used as effectively by humans as that from lk, and the same applies to Ca gluconate or lactate [13, 14]. kroyd and Krishnan [15] gave Ca lactate in a large-scale

experiment to school children in India and reported a sign cant improvement in weight and growth. The availability inorganic salts appears to depend on their solubility. Stegger and Mitchell [16] found that the availability of Ca to man fr dried-milk solids or homogenized milk was the same that from whole milk. Cheese Ca was as good as that fr milk [17].

As far as vegetable Ca is concerned, as a rule this Ca is n so available as milk Ca. Breiter *et al.* [18] found that t utilization of carrot Ca was on an average 13·4 per cen which is lower than the usual figure for milk (about 20 p cent.). The figures they give also illustrate the drawbacks relying on vegetables as the main source of Ca. To obt 202 mg. of Ca, 700 g. of carrots had to be eaten, this givi only about one-quarter of the daily requirement. On the oth hand, lettuce Ca was even better than milk Ca when fed amounts giving the same Ca intake, but here again the content is so low (33 mg. per 100 g.) that, like most vegetabl lettuce could only be used as a subsidiary source of dietary C

*Pasteurization of milk*. A great deal of controversy has rag over this process, and unfortunately considerations other th scientific ones have at times been used as arguments. As far Ca is concerned, its availability is not very much affect Kramer *et al.* [19] found in children and adults that the Ca raw milk was better retained than that of pasteurized mi Ellis and Mitchell [20] put rats on to diets of suboptimal content and found that 98 per cent. of the Ca of raw milk w retained, and 92 per cent. of that of pasteurized milk. On t other hand, Henry and Kon [21] and Auchinachie [22] carri out simultaneously and independently balance experiments rats similar to those of Ellis and Mitchell and found no diff ences in the availability of Ca from these two sources. Expe ments undertaken by Crichton and Biggar [23] showed differences whatever in the nutritional status of calves fed either type of milk. Steggerda and Mitchell found in man [2 that heating milk to 160° F. for 30 minutes did not affect t utilization of the Ca. It seems therefore unlikely that paste

ation materially affects the nutritional value of Ca in milk, nd if any slight change occurs, this is more than counter-alanced by the general improvement in the milk.

*Water as a source of Ca.* Hardness in water is expressed in a ariety of ways, usually as parts of $CaCO_3$ per 100,000 or 000,000. This assessment makes no distinction between the arious salts which can cause hardness, and may be bicar-onates, sulphates or chlorides of Ca, Mg and other metals. or this reason it is very difficult to assess water as a source f Ca. Soft water with a hardness of 15 parts of $CaCO_3$ per illion is obviously a poor source of Ca since it contains only 6 mg. per 100 ml. (if the hardness is all Ca), and a daily take of, say, 4 pints would give only 14 mg. Fairly hard aters would give on this basis a daily intake of about 200 g. [25]. Really hard waters such as are found in the Kenhardt strict of South Africa [26], with a total hardness of 1,000 arts of $CaCO_3$ per million, would give 900 mg. per day if pints were consumed. Walker [27] has calculated from figures vailable in South Africa that the Ca contributed by the drink-g water is negligible in the case of one-third of the popu-tion: that half of the population get about 60 mg. daily from ater; and that with less than one-sixth of the population the a ingested from the drinking water is from 100 to 300 mg. r day. Except in the case of the latter group, the Ca from rinking water makes an insignificant contribution to the daily a requirement, and in the case of the last group it is not nown if the hardness is all due to Ca or how available this a is. It thus appears wise to disregard water as a source of a till more knowledge is forthcoming. Hutchison [28] in his ell-known textbook considers water to be negligible in this spect.

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## CHAPTER III

# Intestinal Absorption of Calcium

As indicated in the introduction, a number of factors a involved in the absorption of Ca in the intestine. We are st uncertain of the quantitative aspect of absorption, althou some ingenious attempts to determine it have been describe It seems quite clear that Ca absorption is not intake min faecal excretion. This assumption has been made by McCan and his school [e.g. 1], and in spite of the criticism of Stegger and Mitchell [2] has been repeated in a more recent public tion [3]. Such a concept is quite untenable. Bergeim [4] show that Ca and P entered and left the intestine at various poin and Nicolaysen *et al.* [5] considered that 0·5 g. to 1 g. of C might be secreted daily in the digestive juices, and if this is it can be seen that such Ca could exceed the daily intake a vitiate any intake minus excretion calculations.

Nicolaysen *et al.* [5] made a series of interesting calculatio of the endogenous Ca loss in the faeces, by which is meant th which does not arise from unabsorbed dietary Ca. Th assumed various levels of secreted Ca and also that the pe centage absorption of this Ca was the same as that of food C Whether this latter assumption is correct is possibly open doubt, but their figures for secreted Ca agreed well with tho calculated by Logan [6]. The results showed that at levels Ca intake between 400 and 600 mg. daily, the absorption food Ca varied between 52 and 84 per cent. and naturally, the figure for secreted Ca rose, so did the apparent efficien of absorption of food Ca. Blau *et al.* [7] using the isoto dilution method of Vissek *et al.* [8] also found the absorpti of ingested Ca in man to lie between 44 and 67 per cent., a

ssek *et al.* using the same method found the 'true digesti-
ity' of Ca in cattle to be from 2 to 56 per cent. Geissberger
has reported similar figures in humans using $Ca^{45}$. These
ures are surprisingly high since, as will be seen, the final
ilization of ingested Ca in man seldom exceeds 30 per cent.
ie calculations of Nicolaysen *et al.* were based on the find-
gs of three adults aged 29 to 58 years. Figures for children
d babies are badly required since it is difficult to see how
ey store Ca on their known Ca intakes unless Ca absorption
extremely efficient.

It would be expected that Ca absorption would take place
iefly high up in the small intestine where the reaction was
ore acid. Bergeim [4] and Adolph and Liang [10] found in
ts by direct analysis that Ca was absorbed in the small
estine, and Bergeim commented on the finding that Ca
sorption occurred chiefly where P secretion was greatest,
d considered this to be an important relationship. Both
colaysen [11] and Robinson *et al.* [12], working with loops
intestine or Thiry–Vella fistulae, found that the rate of Ca
sorption was increased with increased concentrations of Ca
the intestine. Innes and Nicolaysen [13] found that the
moval of the caecum did not affect Ca absorption. Jansen [14]
served that urinary excretion and intestinal absorption bore
relationship to each other, which McCance and Widdow-
n [15] confirmed, finding that the urinary excretion of Ca
llowed the intestinal absorption (intake minus faecal ex-
tion), but not rising or falling to the same extent. They
ggested that changes in urinary excretion might be regarded
a valuable index of changes in intestinal absorption.

Nicolaysen [16] advanced a novel and attractive hypothesis
ich unfortunately has not yet been confirmed [Henry and
on, 17]. He suggested that, at any rate in rats, the intestinal
sorption of Ca was a function of the Ca saturation of the
dy, and this was governed by an endogenous factor which
eded vitamin D for it to act. The factor was present only in
ung animals and this was why vitamin D had little action
the absorption of Ca in older rats. There is no doubt, as

will be seen again later, that the retention of Ca is gover in some way by the level of the body stores and the utilizat of Ca is more efficient with lower body stores, but no one far has demonstrated the mechanism of this action.

*Reaction of the intestinal contents.* On purely chemi grounds it might be expected that Ca absorption would seriously interfered with if the reaction of the intestine beca alkaline. McGowan [18], on the basis of balance experimer has stressed the importance of the gastric HCl in the abso tion of Ca and considered it the chief single factor in t process. The absorption of Fe is greatly reduced in cases achylia gastrica, and Bussaberger *et al.* [19] found that g trectomy in puppies caused severe osteoporosis and even sp taneous fractures. The animals were in negative Ca balan They attributed these effects to the absence of gastric HCl keep Ca salts in solution, the rapid transfer of food throu the intestines in the absence of the stomach and also to pc cibal acidosis which might mobilize Ca from the bones.

The addition of acid to the diet, or giving an acidic type diet, may apparently increase Ca absorption, and alkali in t diet may have the reverse effect. This has been claimed b number of workers but mostly in acute experiments [e Irving and Ferguson (20) or by the use of Thiry–Vella fistu (12, from $CaCl_2$, but not Ca lactate solutions)]. Howev while intestinal absorption may be improved by giving aci in many cases the urinary excretion of Ca was also increas causing in the end a greater total loss of Ca from the bo On the other hand, it has been stated that the reaction of t diet has no effect on Ca absorption, and that alkaline solutic introduced into the intestine rapidly become acid. Similarly has been stated that acid solutions actually depress absorption.

It seems almost impossible to draw any conclusions fr the very contradictory evidence that exists. While acid so tions may for the moment increase Ca absorption, it see probable that the contents of the intestine are well buffer that such changes in pH are rapidly compensated for, and th

a excretion in the urine is increased, the extra Ca being ssibly mobilized as base to neutralize the acid introduced to the body with the food. An exception to this statement regards pH may exist in the case of fat.

*Fats and soaps.* Unabsorbed split fat in the intestine will rm insoluble Ca soaps and thus may interfere with Ca sorption. Such conditions may happen when the flow of e is obstructed. Fats have an antirachitic effect when diets gh in Ca and low in phosphorus are used (like the well-own Steenbock-Black rachitogenic diet), but not with diets gh in P and low in Ca [21]. This is almost certainly due to e precipitation of Ca as Ca soaps, which would equalize the : P ratio with Steenbock–Black types of diets, but make the te of affairs worse with diets of low Ca : P ratio. Jones [22] vestigated the effect of various substances upon the pH of e intestinal contents, since Boyd *et al.* [23] had suggested at fats exerted their antirachitic action by lowering the pH. nes found that while lard, oleic acid and vitamin D lowered e pH to the same degree, vitamin D was much more anti-chitic. Lactose also lowered the pH to the same degree as d, but had no antirachitic action under his experimental nditions, and phosphate, also antirachitic, had no effect on intestinal pH. On the other hand, French [24], working h rats, used diets containing from 5 to 45 per cent. fat and nd that the utilization of Ca fell consistently as the fat ntent of the diet was raised above 5 per cent., which he ributed to changes in the reaction of the intestinal contents, dity being highest with the 5 per cent. fat diet. He thought t besides the reaction of the gut, absorption of Ca was verned by the formation of readily absorbed bile-fatty acid-complexes. Thus under normal conditions it appears that in the diet has within certain limits of concentration an uvant action on Ca absorption, the optimal fat : CaO ratio the diet having been stated as lying between 1 : 0·045 and 0·06 [25]. From the point of view of practical nutrition in mans the question is probably an academic one since Mallon *al.* [26] and Steggerda and Mitchell [27] found that dietary

fat had, within a wide range, no effect upon Ca retentior humans. Humans do not normally eat fat-free diets or d with a fat content above about 20 per cent.

*Protein.* Many workers have claimed that there was association between protein and Ca in the diet, Ca absorpt and utilization being better with high protein intakes. Shern and his colleagues [28] found with rats that the total body was raised during growth by increased protein in the diet, this difference disappeared as the animals grew older. Chinese [29] it was found that a higher intake of prot facilitated the attainment of Ca equilibrium of intake and o put, which is probably of importance in peoples habitua subsisting on low Ca intakes. McCance *et al.* [30] have p duced evidence that Ca absorption (intake Ca minus faecal is dependent on the protein intake, and that such absorpt may fall to very low levels in the absence of dietary prot It has been suggested [31] that amino acids increased solubility of the phosphates and carbonates of Ca. Hall a Lehmann [32] found that after ingestion of a powder ma by mixing peptone and $CaHPO_4.2H_2O$, urinary and serum values rose higher than if the Ca salt was taken alone and s gested the use of this powder in aiding Ca absorption. St gerda and Mitchell [2] have given reasons for doubting interpretation that McCance *et al.* put upon their results, sir unlike these workers, Mitchell and his colleagues did not th that Ca absorption could be simply calculated by subtract faecal Ca from that in the dietary intake.

*Fibre, bulk of stools, &c.* In general it can be said that b or fluidity of the diet has no effect upon the faecal excret of Ca, nor does constipation [33]. Excess fibre in the diet h no effect upon Ca retention in rats, but in dogs the absorpt of Ca was lowered. Johnson [34] studied three patients w ileostomies and colostomies and found that the normal action of the terminal ileum was acid, but an excess of fi in the diet changed the reaction to neutrality, which mi possibly lower Ca absorption.

*Appetite.* A lack of Ca in the diet depresses the appetite, a

e versa [35]. This is well known to all working on Ca me-
olism studies in animals, so that when comparisons of the
ects of additional Ca in the diet are being investigated, it is
ual to use the paired-feeding technique so that the intakes
at least isocaloric between the various animals.

*$CaCl_2$ acidosis.* It has long been known that the ingestion of
$Cl_2$ causes a profound acidosis, and this has been explained
due to a selective absorption in the intestine, the Cl ion be-
absorbed and the Ca excreted in the faeces as $CaCO_3$ [36].
ore recently Robinson *et al.* [12], working with Thiry–Vella
ulae in dogs, have reported that the acidosis caused by
$Cl_2$ is not due to selective absorption, but to the displace-
nt of other cations in the body by Ca, whose subsequent
retion produces a deficiency of total cations.

*Phosphate.* Under experimental conditions excess P in the
t, associated with a low dietary Ca, greatly interferes with
absorption and the blood Ca level falls, the animals may
e tetany and bone formation is severely interfered with,
sing the typical low Ca form of rickets [37]. This condition
lleviated by vitamin D dosage even when the Ca and P
ntents of the diet remain unchanged. This action of P is
ily produced in rats and other experimental animals, but
s less likely that it occurs in humans. A similar clinical
ture, but for quite different reasons, is seen in the condition
al rickets, when the kidney is unable to excrete phos-
te [38], and in women with severe Ca and P deprivation,
after multiple pregnancies and lactations [39]. Human
taries almost without exception contain much more P than
but this imbalance does not affect Ca metabolism. Only
chsenring *et al.* [40] have reported a marked depression of
retention when phosphate was added to the diet. Two
ent studies have shown that the ingestion of large amounts
P as phosphate or as $H_3PO_4$ have within wide limits no
ct upon the Ca balance. Malm [41] gave adult men phos-
ric acid at levels of from 0·8 to 3·16 g. (250 to 1000 mg. P)
y for several weeks. The Ca intake varied from 450 to
mg. per day. No effect at all was seen on the Ca balance.

The same was found when the P was given as a neutral Na- mixture to increase the level of intake of P from 1·4 to 2· daily, the Ca intake being 0·5 g. per day. Lauersen [19 private communication] tested the effects of the daily ingest of 5 g. of $NaH_2PO_4.2H_2O$ or 2 g. of $H_3PO_4$ on human adu In both cases the same was found, no effect upon Ca excret being noted. Baylor *et al.* [42] found the same with a wor who was given 2,000 mg. P as phosphate daily. Nicolay *et al.* [5] quote from their extensive unpublished mate which supports the findings of Malm. It may be conclu on the balance of evidence that quite large increases i ingestion, either as phosphoric acid or neutral phosphate, not affect Ca metabolism in man to any significant extent

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CHAPTER IV

# Intestinal Absorption of Calcium *(continued)* Influence of Phytic Acid

s substance is inositol hexaphosphoric acid and occurs as rge part of the organic phosphorus of cereals and seeds, ly in the free state and partly as phytin, the CaMg salt. significance as far as Ca metabolism is concerned is that it ns an insoluble salt with Ca and thus interferes with Ca orption in the intestine. Quite frankly the present writer s that as stated by Horder *et al.* [1] 'the antagonism of tin to the absorption of calcium is not highly significant' the whole question merits a fairly lengthy description as as caused much stir in certain circles.

Mellanby [2] originally noted that certain forms of cereals an anti-calcifying and rachitogenic power when fed to pies. This finding was confirmed by Bruce and Callow [3], found that the agent responsible was phytin or phytic . They explained its action with high Ca diets by showing the P of phytin was not available, and with low Ca diets howing that phytic acid had a Ca salt at least as insoluble Ca phosphate. In the latter case they thought that excess sphate and excess of phytate acted similarly and equally ne diet in anti-calcifying power. In 1936 Lowe and Steen- k [4] confirmed these findings and demonstrated that the itogenic power of cereals was lessened by the hydrolysis ne contained phytate.

McCance and Widdowson [5] analysed 64 different cereal

foodstuffs for phytin and found the highest values in wh meal. The content tended to vary with the total P cont foodstuffs high in total P usually containing more phytin, this was by no means invariable. Horder *et al.* [1] repo that whole wheat meal contained about 380 mg. P per 10 and white flour about 127 mg. P. In 70 per cent. extrac flour only about 15 per cent. of the total P was presen phytic acid, but in 90 per cent. extraction flour, 55 per c was present as phytic acid P. Thus the phytic acid content sharply as the extraction rate of the flour was increased. Y and Dju [6] analysed various components of Chinese diets stated that 25 to 50 per cent. of the P of a typical Shan diet might be in the form of phytin.

Following the report of Bruce and Callow, work was d to see how phytic acid acts in the diet and what practical c sequences may follow from this. Harrison and Mellanby found that phytic acid had a powerful rachitogenic act equivalent to that of oatmeal on the basis of its P cont Phytic acid P was not unavailable, but acted by precipita Ca in the gut, oatmeal containing enough to render unavail twice the Ca in the meal, or in other words, Ca present in o constituents of the diet. This action could be overcome adding Ca to the diet. Krieger *et al.* [8] found that the C Ca phytate was as available as that of $CaCO_3$, but the phytic acid was not so easily used as that of phosphate [9

These results would have had but an academic interest it not been for the use of high extraction flour in the Un Kingdom during the recent war. This change, when 85 cent. extraction National wheatmeal was introduced, increa the phytic acid content of the flour to virtually double and t there appeared to be grave danger of slow widespread de cification of the skeleton, especially among those habitu eating large amounts of bread.

Experiments to investigate this possibility were conduc by McCance and Widdowson in 1942 [10]. These work carried out balance experiments on 10 subjects, 5 healthy and 5 healthy women. They found that the absorption of

gauged by the intake compared with the faecal excretion, less if the subjects ate bread made from 92 per cent. raction flour than if they took bread made from 69 per cent. raction flour. The bread provided 40 to 50 per cent. of the l calories. The subjects were studied at two levels of Ca ke, about 500 mg. per day, and about 1,200 mg. On the level of Ca intake, the subjects were in negative Ca balance both the white and brown breads, but less so on the white ad. The data for the high level of Ca intake did not indicate t state of balance the subjects were in. This effect of brown ad could be reproduced by the addition of appropriate ounts of sodium phytate to white bread. When Ca as carate or phosphate was added to the 92 per cent. extraction r to bring the total Ca intake to about 1 g. per day, all the ects but two went into equilibrium or positive Ca balance he fortified brown bread. One point of criticism of these eriments as reported is that one does not know the daily intake of the subjects before the experiments began, but umably it was higher than the 500 mg. or so of the first erimental period; the transfer from a high to a low Ca diet oduces complications which will be mentioned elsewhere. ddition, it might be stated that the balance periods were e short. McCance and Widdowson made suggestions as to amount of Ca which should be added to flours of different actions. They favoured $CaCO_3$, as $CaHPO_4$ was visible as e specks in the baked bread, though both appeared equally tive in aiding Ca absorption. Following on this work, Ca compulsorily added to the National wheatmeal in the unt of 7 oz. of chalk per 280-lb. bag.

his work was repeated in part by Krebs and Mellanby [11] human subjects, using bread made from 85 per cent. and er cent. extraction flours. On a Ca intake of 550 mg. per the subjects were in negative balance on both breads, but on that made from 75 per cent. flour. With a Ca intake of mg. per day, Ca equilibrium was attained with the brown d. No details were given of the subjects' normal diet, nor he time allowed for the adjustment of Ca metabolism to

a possibly altered intake, but McCance and Widdowso main conclusions were confirmed. Hoff-Jørgensen *et al.* [ likewise showed in children that high cereal diets interfe with Ca absorption. Henry and Kon [13] repeated the w using rats. The dietary conditions were very stringent, animals getting only white or wholemeal bread with a vita supplement. In spite of this, the retention of Ca from wh meal bread was only slightly lower than that from white bre and the addition of bone-meal or creta preparata to the wh meal bread made surprisingly little difference to the percent retentions, a rise of 4 per cent. at most. The authors stated t their results supported the findings of McCance and Widd son, which was true, but it was slight support.

Early in the late war the Government of South Africa int duced a National wheatmeal of higher extraction (96 per ce than that used in the United Kingdom. In view of the findi in the United Kingdom, the National Nutrition Council South Africa instituted experiments to test the effects of bro bread on Ca metabolism. The results of these experime carried out on three human subjects for 19, 13 and 6 wee respectively, showed that changing from a diet contain 1,000 mg. Ca and a small amount of bread per day, to of about 500 mg. Ca and containing 1 lb. of standard bread per day, caused a negative Ca balance for some wee This balance became increasingly less negative, and finally subjects went into equilibrium or positive balance and e made good their previous Ca losses on this low level of intake. The phytic acid content of the 96 per cent. extract meal used in making the bread was 145 mg. per 100 g. weight [Walker *et al.*, 14].

The reasons for this adaptation were discussed by th writers, who pointed out that the hydrolysis of phytate almost complete when the subjects were on the low Ca d This relationship, an inverse one, between the Ca level of diet and the extent of hydrolysis of phytate, has also b reported by Cruickshank *et al.* [15]. The difficult questio where this hydrolysis occurs. Rats have a phytase in t

stinal secretions which is a real intestinal enzyme and not
lant origin [Marcy, 9]. The same is true of guinea-pigs and
bits, but in man no such enzyme has been demonstrated.
act, the degree of hydrolysis in man appears to vary con-
rably, as judged from various reports on this, McCance
Widdowson [5] finding much less hydrolysis than Walker
*l.* [14] and Cruickshank *et al.* [15]. However, hydrolysis
loubtedly decreases the interference with Ca absorption,
e McCance and Widdowson [16] found that previous
rolysis of the phytic acid of the flour before baking the
ad improved Ca absorption, and that removal of the pro-
ts of hydrolysis improved Ca absorption still more. Thus
his score it must be concluded that humans are able some-
to hydrolyse phytic acid in the intestine when the Ca
tent of the diet is low.

Another aspect of the matter is the finding that substantial
ounts of the phytic acid are hydrolysed by phytase in the
r during baking. McCance and Widdowson [17] found
t the phytase of wheat flour was resistant to dry heat, and
ing at 260°–360° F. did not destroy all of it. Mellanby [18]
nted out that not only the wheat but also the yeast used
he dough contained phytase and that the phytic acid con-
of the loaf could be reduced from an initial value of
mg. phytic acid P per 100 g. to one of 77 mg. while the
gh was standing. The effect was more marked as the pH
lowered towards 4·5. Walker [19] likewise found that 40
9 per cent. of the phytic acid was destroyed during baking,
ending on the process used. Thus it is evident that a great
l of phytate of flour is destroyed in bread-making and that
content in flour is no criterion of that in the final product.
he other point that the South African workers stressed was
ability of the body to adapt to low Ca intake, which was
evident from their figures. This has been noted by other
kers [e.g. Steggerda and Mitchell, 20] and will be discussed
more detail later (pp. 54–56).

ield evidence in this connexion may also be considered.
us Hicks [21] has pointed out that until eighty years ago,

all Europeans consumed wholemeal flour, a number still
and it seemed unreasonable to assume that these ancest
suffered from an adverse Ca balance or had tetany, and t
history carried more weight than laboratory experiments
a few humans for short periods. Walker *et al.* [14] cited e
ence from the literature of people subsisting on high ce
diets with low Ca intakes of about 300 mg. per day, who w
either in positive balance or equilibrium and who should h
been ideal candidates for phytate action.

The chief shot in the locker of the protagonists of
deleterious action of phytic acid is the evidence from E
when almost straight-run flour was adopted during the rec
war, and an increase in the incidence of rickets actu
occurred. The sequence of events is well summarized
Jessop [22]. Between September 1940 and February 1942
extraction rate of the flour was increased from 70–72 per c
to 100 per cent., and in addition the importation of cod-l
oil and other sources of vitamin D fell considerably beca
of the war. No reliable figures exist for the incidence of rick
before the question arose. The records of the radiolog
departments of children's hospitals showed that the incide
of rickets for August 1941 to July 1942 was almost double t
of the period August 1939 to July 1940, and the mothers of
children concerned were emphatic that no change in diet
habits had occurred, the staple diet being bread and butte

A survey was then carried out in the spring months of e
year from 1943 to 1948. The incidence of rickets in children
to 4 years of age rose to its highest figure in 1944 and t
declined. The figure for children 3 to 6 months of age and a
that for those between 7 and 12 months also reached its ma
mum in 1944. The extraction rate of the flour was reduced
85 per cent. in December 1943.

These results can be interpreted in many ways. As Dav
son [23] has pointed out, there was no control experime
The extraction rate was reduced in December 1943, but in s
of that the rate of rickets was highest in 1944 and was still h
in 1945 and 1946. Furthermore, statistical analysis showed t

incidence of rickets was related to an inadequate intake of amin D and of milk. The fact that all the children, including klings, were affected implicates some other common factor n bread. Walker [24] has also pointed out the lack of cor-ation between the introduction of high extraction bread and incidence of rickets, the time lag being 1½ to 2 years. He cluded his article as follows:

Though the increase in incidence [of rickets] may have been influenced by the change in the composition of the staple bread, it is certain that other factors also operated. Saunders [25], for example, considers the increase to have been largely due to the greater scarcity of foods and con-entrates containing vitamin D. Whatever factors were esponsible, the phenomenon can hardly be used as un-quivocal evidence in support of the phytic-acid hypothesis.

The chief contention of the South African workers is that ny of the investigations demonstrating the deleterious action phytic acid have been of too short duration. Thus the more ent experiments of McCance and his school [McCance and lsham, 26; Widdowson and Thrussel, 27] were at most ried out for only 14 days. In fact, had Walker *et al.* [14] pped their experiments at 4 weeks, they would have agreed h McCance and Widdowson [10].

On the other hand, it must be admitted that the supply of min D is much greater in South Africa than in the United gdom, and this may well be an explanation of the differ-es observed, especially since McCance and Widdowson [10] Widdowson and Thrussel [27] have denied that any ptation to low Ca intakes occurred in their subjects. llanby [28] has stated that a high phytate diet demands a h vitamin D intake, and Spitzer *et al.* [29] showed that min D made the P of phytate almost as available as in-anic P. In any case it is not considered in South Africa that tic acid in the flour is of the slightest importance, any Ca s added to flour being included merely to increase the daily intake; and it appears also that in the U.S.A. it is not

considered that there is any danger from anti-calcifying fac in cereals [30].

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CHAPTER V

# Intestinal Absorption of Calcium (*continued*) The Action of Vitamin D

THIS vitamin is the most important single factor in the abso tion of Ca. It is formed from a sterol precursor by ultra-vi irradiation and was originally held to be part of the gro fat-soluble vitamin, later called vitamin A [Mellanby, McCollum *et al.* [2] were able to separate the anti-xeroph mic and antirachitic properties of cod-liver oil by appropr treatments, and later it was found that irradiation of cer foodstuffs increased their antirachitic but not their a xerophthalmic properties.

Steenbock and Black [3], Hess *et al.* [4] and Rosenheim Webster [5] reported independently and simultaneously t the irradiation of cholesterol or phytosterol with ultra-vi rays converted these substances, previously inactive, i powerful antirachitic agents. It was soon clear, however, t the active substance was not cholesterol, but a contamina which subsequent work proved to be ergosterol [Rosenh and Webster, 6; Windaus and Hess, 7]. The active substa called calciferol, differs from ergosterol in having the sec sterol ring opened between C 9 and C 10, and is called $D_2$ ergocalciferol, $D_1$ being a name previously given to a mixt of $D_2$ and inactive isomers and now no longer used.

It was at this time supposed that calciferol was the natu vitamin D, but it was found that it had much less activity t an equivalent amount of cod-liver oil when tested on chick

ss and Supplee, 8; Massingale and Nussmeier, 9], and this to the isolation of the vitamin present in cod-liver oil. This closely related chemically to $D_2$. The precursor is 7-ydrocholesterol, which after irradiation becomes the active min with the opening of the second sterol ring. The active npound is known as vitamin $D_3$ or cholecalciferol. Both and $D_3$ have an equal activity for mammals [$40 \times 10^6$ i.u. g., Smith *et al.*, 10]. $D_3$ is also equivalent to 40,000 i.u. per in the chicken, but $D_2$ has a value of only 1,000 i.u. per mg. ls, 11]. Russell *et al.* [12] and McChesney [13] found that difference was not due to destruction of $D_2$ in the intestine e the same difference was seen if the vitamins were given enterally. Since most research on vitamin D has been ne on mammals, ergocalciferol is the vitamin usually em-yed. A number of other sterols with activity similar to that vitamin D have been described.

rradiation with sunlight has long been known to have an irachitic effect in animals. In fact, since vitamin D does not ur to any significant extent in foodstuffs other than fish-r oils, humans depend to a considerable extent on sunlight their supply of it. Children require the vitamin during the iod of active bone formation, but it is still doubtful if it is ded by adults. The altitude of the sun is the governing factor o its antirachitic power [Irving and Schwartz, 14], and thus higher latitudes children should be given supplements of a ent fish-liver oil during the winter.

Sunlight activates an antirachitic precursor in the sebaceous retions of the skin [Hou and Tso, 15], which can be noved with ether. This activated precursor is subsequently bsorbed into the body. Bourne [16] has suggested that birds en themselves from the preen gland in order to obtain amin D which is present in the preen-gland oil, though nnken [17] considers that in birds, too, the activated vitamin absorbed through the skin. 7-dehydrocholesterol has been lated from pigskin by Windaus and Bock [18], and Helmer d Jansen [19] have found that irradiated human surface skin and also the surface skin fat from irradiated human subjects

possess antirachitic activity. Mackenna *et al.* [20], howe reported that there was no provitamin $D_2$ or $D_3$ in hum sebum and concluded that the provitamin was another, as unidentified, substance. It is possible that provitamin D present elsewhere in the body. Scott *et al.* [21] have dem strated that it is made from cholesterol by a cholesterol hydrogenase, an enzyme in the intestinal wall, the small int tine and especially the duodenum having a high content 7-dehydrocholesterol in the mucosa. Possibly ingested chol terol is changed to 7-dehydrocholesterol in the intestine a then carried to the skin, where it is converted into the act vitamin.

In the absence of vitamin D influence the condition rick develops. This is described in more detail in a later chap but it can be stated at this point that it is a bone disea characterized by certain histological changes, and can caused in rats by drastically altering the Ca : P ratio of diet. In clinical medicine the condition is almost invaria due to lack of vitamin D, since alterations in the Ca an contents of the diet equivalent to those produced experime ally are never met with in ordinary human dietaries.

Vitamin D has a number of activities, one of which is effect on intestinal absorption. Schabad [22] as long ago 1910 found that during rickets there was a big loss of Ca in faeces, and during healing or treatment this loss was grea reduced. Harris and Innes [23] first postulated that the act of vitamin D was to increase the net absorption of Ca an in the intestine. This view would presuppose that rach cartilage would calcify properly if the supply of Ca and P adequate. That this will happen has been demonstrated Robison and Soames, and Shipley *et al.* [24], who showed t rachitic epiphyses would calcify *in vitro* if immersed in flu of appropriate composition.

Smith and Spector [25] found that mineral oil interfe with the action of the vitamin if given simultaneously mouth, the same interference occurring if the animal irradiated; since the oil was not absorbed, it presuma

rted its antirachitic action in the gut. Nicolaysen [26] pro-ed evidence that the vitamin acted specifically on Ca ab-ption, P absorption being secondarily improved, and es [27] found that a hypercalcaemia followed vitamin D dication to rats on a high Ca : P ratio diet and also con-ered the action of the vitamin to be on Ca absorption. He nd that the degree of healing of rickets bore a relationship he blood Ca level. Work using radio-Ca also shows that vitamin increases intestinal Ca absorption in rats [28] and ckens [29]. The exact mechanism of this action is not under-od. Harrison and Harrison [30] have adduced evidence, ng $Ca^{45}$, that vitamin D acts on Ca absorption in the lower t of the small intestine. Nicolaysen [31], as already men-ned, considered vitamin D to be associated with an endo-ous factor which controlled Ca absorption in young mals. Zetterström and Ljunggren [32] have claimed that the min is an activator of the enzyme alkaline phosphatase, ch is widespread in the body and is found in the intestinal cosa. But phosphatase has not yet been implicated in Ca orption.

Apparently there is no evidence that vitamin D acts on P orption. While it is true that addition of P to a low P diet ses rapid healing of experimental rickets [33], this healing dissimilar to that caused by the vitamin. This has been wn by Irving [33], who stated that the histological changes he epiphyses and also in the teeth differed in many respects n the two treatments. Experiments with radio-P have also n undertaken. Dols *et al.* [34] found no change in $P^{32}$ ab-ption when vitamin D was given to normal or rachitic rats. nn and Greenberg [35] and Morgareidge and Manly [36] found the absorption of $P^{32}$ to be little or not at all affected the simultaneous administration of the vitamin to rachitic s. Thus the evidence seems conclusive that P absorption is governed by vitamin D.

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CHAPTER VI

# Calcium Content of the Body and of Various Tissues

**Total Ca.** This has been investigated in animals, chiefly by a number of workers. In the case of man, unfortunately and discrepant figures are available, very few indeed in the of children, and many unreliable ones in the case of adul

*Animals.* The new-born rat is virtually Ca-free. Cox Imboden [1] found that a new-born rat weighing 5 g. conta about 14 mg. Ca. At weaning (21 days) and weighing 48 it contained 313 mg. Ca. The mineral content at weaning fairly constant and females contained more Ca than m Bessey *et al.* [2] found that 99·06 per cent. of the total Ca in the skeleton and teeth at 2 months of age and 99·3 per c at 4 months. The Ca : P ratio of the body changed with the increase in weight, Ca and P contents increasing in proportion of growth $\times 5$, P $\times 7$ and Ca $\times 12$ [3]. After 6 mo of age the Ca and body-weights increased in a parallel ma [Sherman and Pappenheimer, 4]. The atomic ratio of C in the new-born was 3·5; during calcification it rose to 1 : 1 as calcification became complete it became 6 : 5, the final v agreeing with the concept of 99 per cent. of the Ca and f fifths of the P in the skeleton, where the atomic ratio was 3

The most recent study of the Ca content of the animal b is by Spray and Widdowson [5]. In the case of rats, they fo that a large increase in Ca content occurred during the s ling period, after which the rate of accretion gradually fell the mature percentage was reached, which occurred in th at about 100 days. A kink in the curve of accretion at a

lays was presumably due to new feeding conditions follow-
weaning. The Ca : P ratio of the body was 0·91 for the
e rat at birth and rose gradually to 1·75 at 246 days of age.
Ca content of adult male rats was about 1·1 per cent. and
emales about 1·3 per cent. in whole fat-free body tissues.
se figures are considerably lower than those for man cal-
ted on the same basis [Widdowson *et al.*, 6].

pray and Widdowson also gave figures for the rabbit, cat,
and mouse. The accretion figures for rat, mouse and rabbit
similar and all begin life with a very low Ca content. The
difference in the rat is not seen in the rabbit. The pig starts
with a high Ca content, but this subsequently falls in per-
age till the 16th week, after which it rises to figures com-
ble to those of adult rats and rabbits. These authors also
useful information on the Ca : P ratio of the bodies of
e animals.

great deal of work has been done on the effect of dietary
other factors upon the Ca content of the body—most of
work being done on rats. The composition of the new-born
is remarkably constant despite changes in the mother's
[7], but if the mother's diet is poor, many more young are
dead [8]. With drastic changes in the mother, such as
ng her a very high Ca : P ratio diet or after parathyroid-
my, the foetal Ca and P are lowered, while giving vitamin
the mother improved the foetal storage of Ca and P [9].
lost work on the effects of the Ca content of the diet upon
of the body has been done by Sherman and his colleagues
almost entirely on rats. Animals on low Ca diets, and
ted in growth, had total Ca contents higher than that of
nals of corresponding weight but lower than that of animals
orresponding age [10]. If the Ca intake was very low, the
y Ca remained stationary and the animals lived for about
eks, showing at the end of that time multiple Ca deficien-
but not rickets. Addition of Ca to such a diet increased
body percentage to the right figure, though the absolute
unt was too low and the animals now had rickets. Camp-
*et al.* [11] found that the second generation of rats on a

poor Ca diet (0·094 per cent.) could survive but could reproduce and had only three-quarters to four-fifths of normal total Ca content of the body. The addition of potassium phosphate to a low Ca diet accelerated Ca up so that it was actually higher in percentage than the nor figure for that age though still low in absolute amount. With a fixed dietary P content of 0·43 per cent., increasing Ca up to 1·04 per cent. has no effect upon the body Ca; b the P was fixed at 0·73 per cent., the Ca content of the b was increased by extra dietary Ca [12]. Shohl *et al.* [13] fo in much the same way that the P intake was a limiting fa in the control of the Ca content of the body in the rat.

Sherman and Booher [14] investigated growing animal diets containing 0·16 to 0·50 per cent. Ca. Although all animals grew at the same rate and looked equally hea those on the higher Ca intake contained more Ca during growth period, the difference gradually disappearing as rats became middle-aged. In a later paper [15], it was sh that rats on a diet containing 0·64 per cent. Ca reached a of total body Ca which was not attained by rats on a diet 0·35 per cent. Ca till 50 days later. Fairbanks and Mitchell also found that the carcase Ca increased with high Ca cont of the diet.

It thus appears that the total Ca content of the body ca increased by extra Ca in the diet during growth even tho the growth rate and appearance of the animals are unaffe This matter will be discussed subsequently in another nexion, namely, as to whether such an increased Ca conte essential and desirable.

**Ca content of the human body during growth**

*In utero figures.* Fehling [17] was the first to analyse foet Since then many more analyses have been made and re figures are reported by Coons [18], Iob and Swanson [19] Givens and Macy [20]. The last authors found the Ca con to rise in apparently an exponential way when plotted ag age, to a final Ca content of 21 g. at term, the greates

and coming in the last 3 months of gestation. The most nt and authoritative findings are those of Widdowson and y [21], who analysed foetuses and still-born children hing from 200 g. to 4·4 kg. The total Ca rose continually about 2 g. at the first weight to 36 g. at 4·4 kg. body-ht, the rise being linear when plotted against body-weight the foetus weighed 0·4 kg. The percentage of Ca likewise but more slowly, from 0·4 per cent. to 1·0 per cent. on a ree body basis. The Ca : P ratio rose from 1·4 to 1·7–1·8, to progressive calcification, and stayed at this latter figure the foetus weighed about 2·0 kg. With one exception, the entae contained negligible amounts of Ca, 29 mg. at 17 s and 280 mg. at term. The total Ca figures found in iterature for foetuses at term vary considerably since es of different weight were analysed, but there is general ement that the Ca percentage is 0·8 on a whole body .

*content of the body of growing children.* Knowledge of the ontent of the body of the child during growth is of impor-e in assessing the dietary Ca requirements of children, but direct information exists on this subject. Many attempts been made to compute figures, based on the Ca content e new-born child, that of the adult, and various assump-s on the rate of accretion of Ca during various phases of growth process.

he first attempt at this was made by Leitch [22]. Her mptions led her astray, as she arrived at an adult figure ·6 per cent. Ca, which is double the figure found on ysis. Nicholls and Nimalasuriya [23] calculated the Ca ents of the bodies of Ceylonese children partly by analysis ones and partly by assumptions of growth, and got values h less than those of Leitch.

nce then an analysis has been made by Holmes [24] of four rent methods of computation. These were the figures of l [25], Mitchell and Curzon [26], data based on the find-of Venar and Todd [27] and Holmes's own calculations. omparison of the final figures shows how the various

assumptions made can produce different results, and incid ally how little is known of the essential data. Shohl assu that the weight of the skeleton, as a percentage of the body-weight, rose up to 6 years of age and then rema constant, the percentage of Ca in the skeleton gradually r as age increased. Mitchell and Curzon assumed that the g in Ca were a constantly rising percentage of the gains in body-weight and that the weight of the fresh skeleton w constant percentage of the body-weight. Venar and T based their figures upon the weights of fresh skeletons sected from children, and proposed that there was a ris proportionate weight of skeleton up to 6 years of age and a decline. Holmes herself also worked out the Ca accretion the basis that these were almost in the same proportion those of total body-weight. In the case of Holmes's figures Ca gains added up to a figure of 1·6 per cent. in the adu

Holmes has pointed out that Venar and Todd's figures quite out of keeping with what might be expected, since greatest accretion was during the period of least growth. It been suggested that the children they dissected were not mal. The method of Shohl, while looking 'correct' in early took no account of pubertal growth and presumably incre Ca storage during this time. The methods of Mitchell Curzon, and Holmes gave results which might have been pected, since the greatest accretions of Ca occurred during fastest growth. The Ca storage figures obtained from bal experiments in the literature were all much higher than t worked out by any of these methods.

In a later paper Mitchell *et al.* [28] analysed a fresh cadaver and obtained a figure for total body Ca of 1·596 cent. On the basis of this more accurate figure for the a Ca content, these workers reassessed the estimate of the Ca increments during growth. The growth data of M dith [29] were used, and these assumptions were made:

(*a*) That the Ca content at birth is 0·8 per cent. (whole b basis).

(*b*) That that in the adult is 1·6 per cent.

) That the change from the infantile to the adult percent- occurs progressively during growth, but more rapidly n growth is more rapid, and thus the Ca gains were test over the period of 14 to 18 years. This final assump- was based upon findings with growing rats, chickens and bs, and it appeared valid to apply them to humans.

ome of the calculated figures for body Ca were as follows:

| *Age* | *Wt. Kg.* | *Ca content g.* |
|---|---|---|
| 1 | 10·6 | 100 |
| 5 | 19·1 | 219 |
| 10 | 33·3 | 396 |
| 15 | 55·0 | 806 |
| 20 | 67·0 | 1,078 |

Viddowson *et al.* [6] analysed the body of a boy aged 4½ rs who died of tuberculous meningitis after a two-week ess. The body was moderately nourished but rather thin weighed 14 kg. The Ca content was given as a percentage ne fat-free body and on recalculation the total Ca is found e 228 gr. The weight of the boy was considerably less than given by Mitchell *et al.*'s figures (17·6 kg. at 4 years and kg. at 5 years), and the Ca content was higher than chell's figure (219 g. at 5 years). It is unfortunately impos- e to argue from one analysis and that of the body of a very hild.

Mitchell *et al.* [28] comment on the fact that balance experi- t data in the literature showed much higher figures for ntion than those calculated by their method and these ntions integrated to a final Ca content much higher than actually found in the adult body. They gave other reasons considering isolated balance experiment data to be of btful value in arriving at conclusions on this matter, cially since the gross errors of balance methods all tend ncrease the estimated Ca retention.

is evident from the foregoing discussion that the existing rmation on the Ca content of the growing human body is nty and has mostly been arrived at by indirect methods.

Many more chemical analyses will have to be carried ou bodies at different ages before we are in a position to a this information with confidence to the question of requirements.

**Ca content of the adult human body.** Here too it mus admitted that while figures from cadaver analyses exist, m of them are quite unreliable and the subjects analysed w not normal. The uncertainty about exact figures has been il trated by Mitchell *et al.* [28], who quote those given in various editions of Sherman's *Chemistry of Food and Nutri* the values varying from 1·15 to 2·2 per cent.

Nicholls and Nimalasuriya [23] analysed bones from C lonese skeletons and arrived at a body Ca figure of 1·65 cent. for males. More recently three analyses have been car out which are the most accurate to date. Mitchell *et al.* analysed the body of a man of 35 years, weighing 70·55 He died of cardiac failure but appeared normal nutrition The skeleton was 14·84 per cent. of the body-weight and tained 99·0 per cent. of the total body Ca. The percentage content of the body was 1·596. The Ca : P ratio of the w body was 2·07 : 1. Widdowson *et al.* [6] analysed the bodie two men and one woman. Of the two males, one die uraemia and one of infective endocarditis, and the fe committed suicide. The Ca contents in all were about the sa 2·3 g. per 100 g. fat-free weight, Ca : P ratio, 1·77. In the l paper from Mitchell's laboratory [Forbes *et al.*, 30], a m killed as the result of a fall, was examined. He weighed 53·8 but was 23 per cent. below average weight for his age height. The Ca content of the body was 1·907 per cent. fresh weight basis, significantly higher than the result of previous analysis, and 2·40 per cent. on a fat-free basis, w agrees fairly well with the figures of Widdowson *et al.* skeleton was 17·58 per cent. of the body-weight and conta 99·58 per cent. of the total body Ca. The Ca : P ratio o body was 2 : 1. They concluded that the percentage of C the body (fat-free basis) ranges from 1·8 to 2·5 per cent.

he present writer has instanced these figures to show what ent work has been done in this field and the difficulties erent in such investigations. All writers have emphasized difficulty of getting fresh and normal bodies and also the portance of avoiding post-mortem changes, especially desic- on during the dissection and analysis of the tissues. Many re analyses must be undertaken before we have figures able from a statistical point of view.

*Ca content of individual tissues.* A bare recital of the Ca tent of various tissues is not very informative although Ca ys an essential role in many tissue functions. It will be seen m the following figures that in most cases the Ca level ears to be related to that of the plasma but not to be cted much by fluctuations in the plasma level. The exact e of tissue Ca is not known but it would appear to be nd in some fairly stable way [31].

Katz [32] was the first to give accurate figures for tissue Ca, mg. per 100 g. for human muscle and 6·9 mg. for dog scle. Burns [33] found that of rat muscle to be about 7 mg. 100 g.; she also gave figures for other rat tissues and scles of other animals. Rat fat has a very small content, but brain has values of 8–16 mg. per cent. Dog and cat muscle was of the same order as that of rat; frog muscle had 24 mg. per 100 g. The trachea contains 0·5 mg. to 4 mg. cent., the value increasing with age due to calcification of cartilages [2]. The Ca content of skin has been determined by wn [34]. The values per 100 g. dry skin were highest for the bit (50–86 mg.) and lowest for the dog and man (31–59 mg.). ürger and Schlomka [35] found that the Ca content of the man aorta rose with age from 0·05 g. per 100 g. dry weight 6·7 years to 1·6 g. per 100 g. at 76 years. Widdowson *l.* [6] in a recent study found that the Ca of human liver about 6 mg. per 100 g. fresh weight and that of the spleen e about 9 mg. per 100 g. fresh weight. The significance of difference is not clear.

he effect of various factors on the Ca content of tissues has n investigated by several workers. Burns [33] found no

change in muscle Ca after the animals had subsisted o
Ca : P ratio diets, with and without vitamin D, or
Ca : P ratio diets. The blood Ca was lowered by a var
means but the muscle Ca remained unchanged. Injecti
parathormone may have raised the muscle Ca slightly.
*et al.* [36] also found that parathyroid tetany did not
the muscle Ca. Loughridge [37] stated that parathyroide
lowered the muscle Ca, but his normal values were highe
those found by other workers. Haury [38] found that t
content of muscle, calculated on a dry-weight basis, was lo
in rachitic animals. Linder [39] reported in rats that a ra
genic diet raised the brain Ca but left the liver Ca uncha
Acute ergosterol poisoning raised both the liver and bra

The giving of Ca salts apparently has little effect on t
content of tissues [40]. Heubner and Rona [41] found
injections of $CaCl_2$ into cats in acute experiments inc
only the kidney Ca. In chronic experiments the Ca of the
and liver possibly rose.

*Bone Ca.* Most animal work has been done on rats,
mett [42] being one of the first to produce accurate ash fi
Gaunt and Irving [43] found the following for the com
analyses of one femur, tibia and fibula (dry, fat-free) o
70-day rats:

| *Ash %* | *Ca % in ash* | *P % in ash* |
|---|---|---|
| 62·77 | 37·14–38·09 | 18·09–18·78 |

The ash content of normal rat bones (dry, fat-free) ros
age as follows [44]:

| *Age (days)* | *Sex* | *Ash %* |
|---|---|---|
| 21 | M | 48·51 |
| | F | 47·06 |
| 40 | M | 54·75 |
| | F | 54·91 |
| 70 | M | 62·77 |
| | F | 64·65 |
| 100 | M | 64·84 |
| | F | 65·25 |

The adult figure is attained by 100 days.

he analysis of separate human bones has been carried out a number of workers. Mitchell *et al.* [28] gave a review of older findings, and Booher and Hansman [45] reported res for the Ca of the tibiae of normal full-term foetuses. ures for the ash of bones of adults have been found by ous workers to be as follows: Ca: 35·09 to 38·80 per cent., P ratio 2·19 : 1 to 2·35 : 1. Mitchell *et al.* [28] analysed left tibia, ulna and ninth rib of their cadaver. The ash and varied from bone to bone, the ash being highest in the a (66·34 per cent.) and lowest in the rib (57·54 per cent.). Ca content of the ash was more constant, varying from 6 in the tibia to 39·37 in the ulna. These variations show unreliability of drawing conclusions about the total Ca of body from the analyses of one bone as Nicholls and alasuriya did.

orbes *et al.* [30] have given the most recent figures for an bones. As in the previous report from this labora- [28] the analyses of individual bones varied considerably. following figures were obtained on fat-free bones:

| *Bone* | *Ash %* | *Ca % of ash* |
|---|---|---|
| Tibia | 69·30 | 37·56 |
| Tenth rib | 52·10 | 36·99 |
| Whole skeleton | 56·89 | 40·09 |

pite of wide variations in ash content from bone to bone, Ca content of the ash appeared to be fairly constant. But ash content of the same bone in different bodies appears to quite variable.

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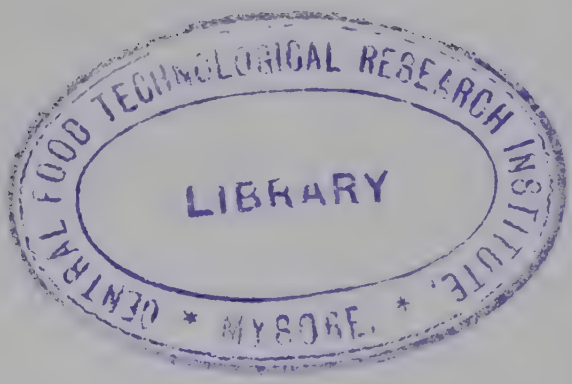

CHAPTER VII

# Utilization and Retention of Calcium in the Body

FORE going into the question of Ca retentions and requirements it is necessary to consider the endogenous Ca loss, the ect of the previous dietary Ca intake, and adaptation to low intakes.

The following definitions are usually conventionally employed in these fields. They have arisen largely as a result of e work of H. H. Mitchell and his school and have found neral acceptance because of their convenience:

*Utilization.* This is applied to the Ca of one particular item the diet, usually under test for availability, and is expressed a percentage of the intake. It is calculated by a special mula.

*Retention.* This applies to all the Ca ingested compared to al excretion and is usually but not invariably expressed as ercentage of the intake.

*Endogenous Ca loss.* By this term is meant Ca arising from ocesses other than those involved in growth, and such a loss curs in the faeces and also in the urine. It is sometimes red to as the 'maintenance requirement'. Exactly how it ses is not yet completely understood. Possibly a constant nover in bone as found by Smith and Smith [1] may contribute to it. It is well known that the kidney does not hold ck Ca during periods of Ca lack as it does, e.g., Na [2]. is and Mitchell [3] considered it might be solely due to

unavoidable leakage through the kidneys and intestinal w
caused by physico-chemical processes.

Hamilton [4] first raised the question. He pointed out t
normal infants excreted about 200 mg. of CaO per day i
spective of intake or weight. He therefore considered that t
Ca must be used for some process of internal metaboli
other than calcification, and if the intake fell below this le
then calcification suffered, not the internal metabolism. Ba
*et al* [5] investigated a number of subjects on low Ca inta
and who were all but one in negative Ca balance. From
results obtained they suggested that Ca 'katabolized' was
available for use again and was excreted. Even in the case
a pregnant woman on a low Ca intake, the Ca loss was
same as if she had not been pregnant, suggesting that the
lost was not available to the foetus. Pugsley [6] found that
increased Ca excretion which followed weaning in the mot
rat could be abolished if the mammary tissue was removed, a
commented on the fact that the Ca absorbed from the invol
ing gland could not be immediately used in the animal's bo
Bell *et al.* [7] considered that the removal during growth
Ca from the inside of the bone-shaft and redisposition o
on the outside formed a kind of endogenous process going
continually.

Mitchell and his school have made an intensive study of t
problem. Ellis and Mitchell [3] and Fairbanks and Mitch
[8] compared the Ca retentions of young rats on diets w
different Ca contents. From their results they concluded t
there was no Ca requirement for maintenance in grow
animals, the Ca being all used in growth and calcificat
processes. The same conclusion was reached by Kinsman *et*
[9] using an ingenious calculation. Their experiments w
actually undertaken to test the utilization of the Ca of milk
pre-school children. Mitchell and McClure [10] had argu
that if there were an endogenous loss of Ca this would app
to make the retention of Ca less at lower levels of inta
compared with that at higher levels, since the Ca lost
lower levels would come partly from unabsorbed Ca a

tly from the body itself. To overcome this, Kinsman *et al.* d this formula to work out the degree of utilization of the k Ca:

$$\frac{\text{Retention for period A} - \text{retention for period B}}{\text{Intake for period A} - \text{intake for period B}} \times 100 = \text{percentage utilization}$$

iod A being that of higher Ca intake and B that of lower ake. This method enabled the authors to see if there was endogenous loss, provided that this loss was not affected the Ca intake, and provided further that the Ca intakes in two periods did not exceed the minimum requirement for ximum retention. The figures they gave showed that there s no endogenous loss of Ca or maintenance requirement in ldren, since the percentage retentions in both periods were same and agreed with the percentage utilization figure of milk Ca. One example from their paper gave the reten-ns in periods A and B as 19 and 20 per cent., and the ization of the extra Ca as 18 per cent. Had there been an logenous loss of, say, 50 mg. per day, the figures would e been 11 per cent. for period A and 6 per cent. for period the utilization, of course, remaining at the same figure of per cent. This demonstrates the validity of their formula l shows clearly that, as with growing rats, there is no endo-ous loss of Ca in children. Ellis and Mitchell [3] say 'in young growing animal these "used" ions as well as the cium coming directly from the alimentary tract may be orbed and retained by the developing bones and the wing tissues at such a rapid rate that, at low levels of Ca ding, the threshold of excretion by the kidneys and the stines is never reached.' Kinsman *et al.* also discussed the ole question at some length.

n adults there is undoubtedly an endogenous loss of Ca l many methods have been used to calculate this. Mitchell l Curzon [11] reviewed the whole subject and compiled a ph from 139 average observations in the literature re-ng intake and output of Ca. A straight line was fitted to

the data by the method of least squares and possessed formula:

$$y = 0{\cdot}6826x + 3{\cdot}0940$$

where $y$ was the Ca output in milligrams per kilogram bo weight per day, and $x$ the intake expressed in the same w The line intersected the Ca output abscissa at 3·0940, wh represented the endogenous Ca excretion when there was Ca intake. This figure would be about 200 mg. per day fo 70-kg. man. Steggerda and Mitchell in a more recent pa [12] found the endogenous loss to be 3·72 mg. per kg. bo weight per day. The calculations of Nicolaysen *et al.* have already been referred to. Using their assumption a Ca secreted and reabsorbed in the intestinal tract, total en genous losses of from 261 to 499 mg. per day were obtai (faeces and urine), the figure rising with higher assu figures for unabsorbed digestive juice Ca. Unfortunately weights of the subjects were not given, but the present wr feels that these figures are on the high side. Blau *et al.* used the isotope dilution method of Visek *et al.* [15], assumed that the specific activity * of faecal endoger $Ca^{45}$ is the same as that of the serum $Ca^{45}$. Visek *et al.* worked on cattle and they found that the faecal endoger Ca did not rise with increase of Ca intake but seemed be lower in animals on a low Ca diet. Blau *et al.*, worl with two human subjects, found a daily endogenous fa loss of 91 mg. in one and 117 mg. in the other, figures the whole lower than those of Nicolaysen *et al.*

It is possible, by means of a back calculation using formula of Kinsman *et al.*, to work out the endogenous of adults from Ca balance figures. From findings of Stegg and Mitchell [16] a figure of 163 mg. per day or 2 mg. kg. body-weight is obtained. Calculations from the figure Owen [17] and Owen *et al.* [18] gave similar results. In

* $\dfrac{Ca^{45}}{Ca^{40}+Ca^{45}}$

bject on a low Ca intake, the daily endogenous loss was
2 mg., or 1·8 mg. per kg., but on higher intakes this rose
over 200 mg. in the same subject. On more liberal intakes
e endogenous loss was higher still. If the assumptions made
e valid, the calculations made from Steggerda and Mitchell's
ures and those of Owen show that the endogenous loss is
t independent of the Ca intake as had been thought. This
in fact implied in the statement in Holmes's article [19]
at 'during periods of generous intake, endogenous losses
uld appear and a maintenance requirement for calcium,
the usual sense of the term, would develop'. One can thus
nclude that the appearance of 'endogenously' derived Ca
the excreta depends largely on the avidity of the body for
. In growth, this demand prevents such an appearance, but
adults, when Ca is not so readily taken up by the body, the
eshold of excretion is exceeded. The adult body seems, how-
er, to have lost the ability of the growing body to react this
y in emergencies, as seen, for example, in lactation, when
ch Ca may be lost. When the adult is in Ca equilibrium
daily Ca intake merely has to satisfy this endogenous loss.

*Effect of previous dietary intake.* It has not been sufficiently
lized by workers using balance metabolic methods that the
ults of such methods must be carefully assessed before
nclusions are drawn, and in particular the previous Ca in-
e must be known and the length of time the subject has
en on this level. If this is not considered and a group of
ople are chosen at random and put on to the same Ca level
intake, it may well be that half will go into positive balance
d half into negative balance, and it would be assumed that
ey had quite different Ca requirements.

This question was first raised by Fairbanks and Mitchell [8]
d more convincingly by Rottensten [20], who found that
e Ca retention of rats depended on their previous Ca level
intake. Rottensten had one group of animals on a level of
5 per cent. dietary Ca and another on 0·8 per cent. Ca.
eir retentions were 85 per cent. and 50 per cent., respec-
ely. Paired feeding was employed and growth was the same

in both groups. After 4 weeks, all rats were put on to a
containing 0·4 per cent. Ca for 5 weeks. The animals
viously on the low Ca diet now retained about 70 per c
Ca and the retention of the others remained at 50 per c
The animals were indistinguishable in weight and appeara
and had they been investigated by someone ignorant of t
previous dietary history it would have been concluded
they were two quite different types of animals. Similar f
ings have been reported by Carlsson [21] using $Ca^{45}$.

Experiments by Owen [17] and Owen *et al.* [18] h
demonstrated the same in humans. Owen [17] studied a gr
who were in balance on 520 mg. Ca per day and who
been attending a diabetic and obesity clinic so that their
vious dietary history was well known. Owen *et al.* [18]
vestigated indigent men whose dietary history was also kn
and who were found to be in equilibrium on a Ca intak
240 mg. to 280 mg. per day. When their intakes were ra
to 550 mg. per day they went into positive balance
stored Ca. Thus here too were two different groups of i
viduals whose metabolic balance behaviour was quite di
ent and had been conditioned by their previous dietary hist

*Adaptation to low levels of intake of Ca.* It can be conclu
that the body has considerable ability in this respect, in s
of statements to the contrary.

The first quantitative findings supporting this concept v
those of Kelly and Henderson [22] and of Kung and Yeh [
who reported that Africans and Chinese were in Ca eq
brium on extremely low intakes of Ca without apparent
pairment of health. Nicholls and Nimalasuriya [24] found
same in Cingalese and suggested that an adaptation to a
Ca intake had occurred. That this adaptation was not uni
to races other than white people had also been shown
Owen *et al.* [18] in the paper quoted above. Kraut
Wecker [25] emphasized the power of adaptation posse
by the body to low Ca intakes and Walker *et al.* [26] sho
by balance experiments carried out over long periods
not only could adaptation occur but Ca losses could be m

while the subject was still on the low Ca diet. The total loss while adaptation was occurring was in one subject ly 2 g.

One of the most important recent papers in this field is by egsted and his colleagues [27], who studied prisoners at ma, Peru. These writers found that intakes as low as 100 to 0 mg. Ca per day were adequate for equilibrium, the re- irement being related to the Ca reserves and to the previous a intake. Similarly, Nicolaysen *et al.* [13] in long-term ex- riments have shown that 13 out of 14 adult male subjects lapted to low Ca intakes in from 2 to 8 months.

The chief antagonists to the theory of adaptation are the cCance school. In their phytic acid paper [McCance and iddowson, 28] they denied that any adaptation had oc- rred, and in a later publication [29], in which some Germans d been studied on low Ca intakes, they virtually poured orn on the whole concept, but did admit that 'it was pos- ble that they [the subjects] had been eating at home amounts milk and cheese, additional to the official rations, which ey did not disclose'. As usual, the balance periods were ort. More recently McCance [30] has reiterated his views, th little additional evidence, including an account of an tack of tetany which he claimed that he himself had after ting a brown-bread diet for a fortnight [31].

One can conclude that the vast majority of workers in this ld feel with Steggerda and Mitchell [32] as follows:

> It appears that in the presence of an inadequate supply of any nutrient, including calcium, the body can adjust itself to the situation, either by a more economical use of what little is available, or by a lowering of its own requirements, so that eventually it comes into equilibrium with the limited food supply. Only during this adjustment period can the body be adjudged undernourished, since only in this period is the body suffering a loss of nutriment. When adaptation is complete, the body replaces from its restricted supply all losses of the nutrient from its body, and unless some

> subsidiary ill effects ensue, it may reasonably be regarde
> adequately supplied with food.

In a classical paper dealing with the whole question of a
tation to undernutrition, Mitchell [33] added:

> If I were to edit this statement to-day, it would be onl
> safeguard against a possible implication that the body
> adjust to any nutritive level, however low. Obviously,
> justment is possible only within certain limits at pre
> unknown.

It seems clear that the mechanism of adjustment is large
function of the level of the Ca stores in the body, Ca b
utilized more efficiently as the stores become depl
[Rottensten, 20]. Rottensten himself pertinently stated:

> In this connection one might naturally ask if it is desir
> to keep the Ca stores filled. Our present knowledge is
> sufficient to answer this question, but it does seem li
> that Ca stores may be considerably reduced, and Ca uti
> tion consequently improved without any harmful effec
> the individual.

*Lactose.* The interesting influence that this sugar has or
retention of Ca should be mentioned at this stage. This i
ence appears to be specific for this sugar and is not share
any other carbohydrate.

Parathyroid tetany is alleviated in animals [34] and hun
by lactose [McCullagh and McCullagh, 35]. Mills *et al.*
found with children that lactose in the diet increased the
retention to a marked degree (33·5 per cent. on an avera
these children excreted less Ca in the urine while taking
tose, and Mills *et al.* considered that lactose exerted its ef
not by rendering the dietary Ca more absorbable in the i
tinal tract but by making the absorbed Ca more utiliz
This view is contrary to that of Bergeim [37], who attrib
the action of lactose to an increased acidity of the intes
contents. Jones [38], however, found that lactose had no

hitic effect in his experimental conditions and made the estinal contents no more acid than did lard, and Outhouse *al.* [39] found likewise that lactose had no effect on intestinal .

Not only is retention increased by lactose, but in addition e bone ash is increased [Outhouse *et al.*, 40], as is the body n. Ca and P [Mitchell *et al.*, 41]. French and Cowgill [42] und the retention effect to occur only in young rats and gs.

Duncan [43] has drawn attention to some significant recent ork by Fournier which he has interpreted to mean that lac-se plays a specific role in the function of the bone cell. If is is so, the small amounts absorbed unchanged must be sponsible, since galactose is apparently inactive in this re-ect. Fournier [44] quoted results showing that lactating rats tting 12 per cent. lactose in their diets stored Ca during the perimental period, while those with starch instead of lactose st a large amount of Ca. Fournier suggests that lactose be-ngs to a group of 'structural' carbohydrates, in contradistinc-on to other sugars which he calls 'energetic' carbohydrates. As uncan states, 'if the effects of lactose on the serum Ca in tany as well as those on bone composition be taken into count, a wider concept of its role than that suggested by ournier emerges, namely, the possibility that lactose can take rt in the biochemical processes fundamentally concerned in lcium metabolism'. Arguing teleologically, this may well be e reason for the presence of lactose and not some other sugar milk, and when cows' milk is modified for infants lactose ould be added to it.

*Ca utilization in adult humans.* Unless on a previously poor a intake, no Ca retention occurs in adults. Ca utilization as been studied by a number of investigators, especially eggerda and Mitchell and their colleagues. Subjects with a reviously normal Ca metabolic status were employed. Steg-rda and Mitchell [16], working with a normal adult man, sted the utilization of Ca in milk and Ca gluconate and und it to be 20 per cent. in each case. In two later papers

working with male subjects [32, 45] utilizations of 18 to 49 p cent. and 16 to 45 per cent. were found, averaging 29 p cent. and 32 per cent.; in both papers the Ca of milk and various milk preparations or Ca salts were investigated. In still later paper they found in 13 subjects a much high average for utilization of milk Ca, 50·2 per cent., but we unable to explain this difference [12]. Geissberger [46], usi $Ca^{45}$, found a utilization of 30 per cent. when taken by mou in the form of gluconate, but a much higher apparent utiliz tion if the radio-Ca was given intravenously, due probably a low level of intestinal absorption of the oral radio-Ca an to exchange of the parenteral radio-Ca in the bones. Patto and Sutton [47] compared the utilization of Ca in the form of lactate, gluconate, sulphate and carbonate by young co lege women. The utilization was the same in all, about 18 pe cent. Breiter *et al.* [48] drew attention to what is a probabl explanation of many discrepancies, that there are high an low Ca utilizers. They found among seven subjects that on group utilized 30 to 35 per cent. of milk Ca and the rest onl 15 to 20 per cent. This aspect has also been stressed b Nicolaysen *et al.* [13], who reported that individuals on constant Ca intake, and observed over long periods of time abruptly changed their retentions and went for no apparen reason from positive to negative balance and back again, th changes being chiefly caused by alterations in faecal excretion Thus, as has been stressed above, in investigating this aspec of Ca metabolism, the need for long studies is imperative since the individual's Ca status is probably always changing The reasons for these changes in retention are not yet known

When subjects on insufficient Ca intakes are tested for th utilization of extra Ca, much higher utilization figures ar found. Recalculation of the figures of Owen *et al.* [18] showe that with their subjects on low Ca intakes, the utilization o extra Ca varied from 44 per cent. to as high as 71 per cent

*Ca metabolism in old people.* Senile osteoporosis is so wel known that many investigations have been undertaken to de termine its cause and possible prevention.

Owen *et al.* [18] found that old men who had been subsisting
a long time on Ca intakes of about 250 mg. per day, and
me of whom had osteoporosis, were in Ca equilibrium, and
ien the Ca intake was increased, went into positive Ca
lance, and thus had not lost their ability to store Ca. Un-
rtunately the experiment could not be conducted for a suffi-
ently long period to see if the osteoporosis disappeared.
milar findings have been reported by Nicolaysen *et al.* [13],
no in long-term experiments found that elderly men stored
a on an intake of 900 mg. per day, indicating a probable
evious Ca deficiency, but also indicating that these old men
o could still retain Ca if they got enough. Vinther-Paulsen
9] likewise found in old patients the incidence of senile
teoporosis to be related to the level of Ca intake.

Cooke [50], however, concluded that it was doubtful if
alnutrition was a common agent in the production of osteo-
rosis, since Ca or P or vitamin D deficiency should produce
teomalacia. There is no doubt that many other factors be-
les Ca deficiency can cause osteoporosis. Immobility is a
ll-known cause, producing a large Ca loss in the urine,
espective of the Ca intake [Howard *et al.*, 51], and the
activity of elderly people may cause the bony changes. Endo-
ne effects must also be considered, though little is known
out this in old age. Albright *et al.* [52] reported post-
enopausal osteoporosis in women, and from analogy of the
tion of oestrogens on bone in birds (see p. 106) tried
strogen therapy. They found strongly positive Ca balances
d after a long interval an improvement in the X-ray appear-
ces of the bones [Albright, 53]. It should be pointed out
at the X-ray diagnosis of bone changes is extremely crude,
at least 30 per cent. of bone salts must be lost before a
ange can be detected [Babaiantz, 54].

Thus it is not easy to conclude whether senile osteoporosis
due to Ca deficiency, or to an altered mode of life, or to an
docrine imbalance, or possibly to all of these combined. It
undoubtedly an increasingly important aspect of geriatrics.

**Ca retention and utilization in children**

*Retention.* This whole subject has been well summarize Holmes in her article on Ca requirements during growth The dietary Ca retentions of infants are relatively high. A [55] concluded that the Ca of breast milk must be very ne completely retained for growth, and Telfer [56] observe retention of 60 per cent. of milk Ca by breast-fed infants will be seen, a high retention must be expected for infant get enough Ca to supply their daily requirements. In o children, the percentage retentions are seldom more than per cent. and usually less. Kinsman *et al.* [9] and Outho *et al.* [57], working with pre-school children, found figure about 20 per cent. Watson *et al.* [58] studied pre-sch children at two levels of Ca intake, 1,122 and 775 mg. day. The retentions were higher in absolute amount on higher level of intake, but the percentage retentions w about the same, being in both cases unusually low, around per cent. Even with a poor previous dietary history, Dan [59] found that 3 pre-school children retained only 17 18 per cent. when the Ca intake was about 1,150 mg. day. Johnston *et al.* [60] found that girls aged 13–14, know have been on high Ca intakes, retained 26 per cent. of intake of Ca. McLean *et al.* [61], in a long study in wh precautions were taken to see that the previous dietary hist did not complicate the findings, found the average retent in boys aged 2½ to 6 years to be 17 per cent.

Calculated figures for the daily retention per kilogram body-weight are given by Mitchell and Curzon [11]. Th show that the amount, which is very high in early life (100 at birth), falls considerably in the first 6 years to 3–4 mg. rises during the second period of rapid growth, from the to the 16th year, to about 6 mg., finally falling as adultho is reached. More recent calculations [Mitchell *et al.*, 62], ba on the analysis of a male subject, were essentially the sa The figures integrated to the adult Ca content. Sherman Hawley [63] had summarized the information in the literat

to 1922 and reported figures higher than those of Mitchell d his colleagues. Almost all the balance work on children s given results higher than Mitchell's figures. Thus Watson *al.* [58] found in children between 38 and 55 months of age ily retentions of 4·6 to 10·8 mg. per kg., the values of itchell *et al.* [62] being between 2·5 and 4·2. Outhouse *et al.* 7] investigated children aged from 3¾ to 6 years. By recal-lation of their percentage retentions it is seen that the chil-en retained from 6 to almost 9 mg. per kg. daily. Children ith a poor previous dietary history would, as might be ex-ected, retain more. Thus three children aged from 3¼ to most 5 years of age studied by Daniels [59], and who had tarded skeletal development, retained from 10 to 11 mg. r kg. A severely malnourished child of 3½, investigated by earne and Moore [64], retained from 50 to 68 mg. per kg. the three periods of study when put on to an adequate et. Daniels [59], from her studies of the three skeletally re-rded children and the fact that they gained weight at a rate ster than normal, considered that her figures and those of milar magnitude in the literature were abnormally high and dicated that the subjects studied had suffered from a pre-ous Ca lack. This would explain the findings of Hunscher *al.* [65] and Jeans *et al.* [66], who found that as the Ca in-ke rose, the retention increased. Macy, in her well-known udy [67], investigated two groups of boys. One group, which d been on a generous mixed diet with a quart of milk per y for three months before observation, retained 103 mg. of a (in absolute amount) per day, while a comparable group on e same diet, but without the extra milk, retained 473 mg. of a. The children studied by Macy [68] had in general low tentions of Ca in milligrams per kilogram body-weight, d they had had a generous Ca intake for the previous 5–6 ars.

It has been suggested that other factors can vary the reten-on of Ca. Thus Johnston [69] found that the percentage tention of Ca could be increased in girls by increasing the tamin D intake, and Holmes [19] has queried whether the

children studied by Kinsman *et al.* [9] got enough of vitamin.

Many of these findings illustrate the unreliability of isol balance methods in children. The Ca retentions as calcul by Holmes were in general much less than those found balance experiments, and as noted above, the figures balance experiments often integrate to a final figure in ex of the adult Ca content. For this reason, many of the repo findings must be rejected.

*Utilization of dietary Ca.* As would be expected from w has been said above, the utilization of extra Ca is the s as the figure for the retention of total Ca [Kempster, Kinsman *et al.*, 9].

Thus in both children (except possibly infants) and adu Ca utilization is seen to be surprisingly inefficient, compa with rats who may retain 100 per cent. of ingested Ca. Mitchell and Curzon stated in 1939, 'quantitative informat on the extent of these wastages of dietary calcium in b human and animal nutrition is woefully inadequate'. T statement is still true.

**Pregnancy and lactation**

The combined processes of pregnancy and lactation impose a severe strain on the maternal organism, especiall the dietary intake is poor. This applies particularly to owing to the Ca content of the maternal milk. Thus Goss found that 60 to 70 per cent. of the total Ca of the bodies rats could be withdrawn by repeated pregnancies and la tions coupled with a low Ca diet. The Ca of the hun mother's milk may represent from 15 to 41 per cent. of total intake [72] and repeated pregnancies and lactations n cause a lowering of the blood Ca to 6·9 mg. per 100 ml serum, with positive Chvostek and Trousseau's signs [73].

*Pregnancy.* From the point of view of Ca metabolism, pr nancy has but a slight effect on the healthy organism. In case of the human, calcification of the foetus demands 35 g Ca at the very most, which means a drain of about 3·5 per ce

om the maternal stores, supposing that no extra Ca was ven in the diet. In the case of the rat, rabbit and mouse pray and Widdowson, 74], the newly born animal is virtually a-free and its calcification imposes little strain on the mother. ne same generally applies to women if the intake is adequate, ough great variations occur [75]. Left to themselves, women nded to choose a diet with a high Ca content [76].

The first and classical balance investigation was carried out Hoffström [77], who studied a woman from the 17th week term. The Ca balance was positive most of the time, just ough Ca being stored for the needs of the foetus. Many orkers have reported, on the other hand, that at the end of station the maternal organism has stored far more Ca than eded for the products of conception. Thus Goss and Schmidt 8] found that rats stored during pregnancy four to five times much Ca as was contained in the foetus, placenta and erus. Even if the young were resorbed owing to vitamin E ficiency, this extra storage of Ca still occurred. Forbes *et al.* 9] found a rapid storage of Ca at the end of gestation in ws and Landsberg [80] has reported the same for women. ummel *et al.* [81] carried out balance experiments on a imipara over the last 65 days of pregnancy, and who, on a eral Ca intake, retained more than twice the Ca needs of the etus. In this case, the patient had an unsatisfactory dietary story and the authors were inclined to attribute the high Ca orage to replenishment of maternal stores. They contrasted is woman with a multipara they had previously investigated no retained, as in Hoffström's case, just enough Ca for the etus. Coons and Coons [82] studied a case from the 31st to th weeks of pregnancy and also found Ca storage at this wer level. Thus there appears to be considerable variation the storage of Ca at the end of pregnancy, and since the evious Ca intake has not always been considered, it is quite ssible that this may have been insufficient, especially as the intake is often deliberately increased during pregnancy. It ems safe to conclude that any extra storage of Ca in the other's body during pregnancy is due to previous deficiency,

and if the Ca intake has been adequate, Ca storage does not occur.

It has been shown that $Ca^{45}$ moves easily across the placenta and a higher percentage of $Ca^{45}$ was found in the foetal than in the maternal bones [Shirley *et al.*, 83; Plumlee, 84].

The Ca intake is however important for the fertility and health of the mother, apart from the Ca requirement of the foetus. Thus rats on a low Ca intake were either infertile or ate their young, or the young died *in utero* [Toverud, 85; Macomber, 86]. Davidson [87] found with pigs that a low Ca diet greatly increased the number of piglets born dead. This is probably not a specific Ca effect, since any type of inadequate diet would presumably interfere with fertility and pregnancy.

*Lactation.* From the point of view of Ca metabolism, this imposes a severe strain on the mother. Most experimenters with rats have found them consistently in negative Ca balance. Sherman and Quinn [88] found that Ca and P were lost together in lactation and subsequently regained in the same ratio, suggesting that they were both mobilized from bone. Direct analysis by Warnock and Duckworth [89] showed that the 'ends' and cancellous parts of the bones contributed this Ca, the shafts being unaffected. Goss and Schmidt [78] found that the largest litters caused the greatest losses, as much as 20 per cent. of the body Ca being removed from the mother rat. They noted that while the animals appeared to get enough Ca in the diet, they were unable to absorb it fast enough. Kletzien *et al.* [90] found that with a diet containing 0·6 per cent. Ca, the blood Ca was lowered, and suggested that mobilization was unable to keep up with the demands of the mammary glands.

Cows can apparently be kept in Ca equilibrium during lactation if the intake is enough [91, 92], but as a rule, even with apparently adequate supplies, they are in negative Ca balance [93, 94]. Both Forbes *et al.* [80] and Huffman *et al.* [92] found that cows which were in negative Ca balance early in lactation, or during the height of lactation, could make this up afterwards, by retaining Ca again. Forbes *et al.* considered

he Ca of the bones was more available for mil
han that of the diet and doubted whether supplemental
the diet was used at all for lactation purposes, though
man *et al.* showed that Ca utilization was more efficient
g heavy milking.

ork on lactating women has shown that these findings also
to them. Donelson *et al.* [95] and Hummel *et al.* [96]
ted negative balances during lactation, but if the Ca
e is adequate, Ca can be retained [97], the retention being
st with the greatest milk production. In this report the
takes were from 1·92 to 2·18 g. per day. Shukers *et al.* [76]
d that lactating women, allowed to choose their own food,
ased their Ca intake 62 to 88 per cent. over that during
nancy, the daily intakes being from 2·03 to 5·3 g., far
er than usually taken. Hunscher [98] investigated three
ting women and found that in spite of large intakes the
tivity of the Ca balance increased after 6 months' high
production, the Ca being lost mainly in the faeces, and
ct to such an extent that the faecal loss sometimes ex-
ed the whole intake. Here, too, the bone Ca was presum-
more available than the food Ca. In late lactation,
less milk was secreted, Ca was again stored in the
.

ese facts illustrate the remarkable elasticity of the body in
ng with emergencies in Ca metabolism. During periods of
lemand, large amounts can be given up, to be regained
when the need falls. But presumably the endogenous loss
t reduced. Whether it is physiological for such large losses
e sustained is another question. It must happen in the
rity of cases that provided the stores are well filled at the
of pregnancy, and provided further that they will be
nished after lactation has ended, no harm ensues. In
ase of women starting off with low reserves, on the other
, it is obvious that serious results may follow, as they
probably have little opportunity of refilling their stores,
cially if subsequent pregnancies follow rapidly. It is thus
ramount importance that extra Ca be included in the diet

of poorly nourished lactating women, and a wise precaution in the case of those better nourished.

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## CHAPTER VIII

# The Dietary Requirement of Calcium

HE calcium requirements of man have been the subject of a eat deal of work and thought, and the subject has been cently discussed by Stearns [1]. The older investigators, who ere ignorant of many of the peculiarities of Ca metabolism, rived at figures which were in many cases incorrect. Sher- an [2], studying balance figures from the literature and from s own observations, supposed that if the subject was in gative balance, the addition of the Ca lost would bring him to equilibrium, and if the Ca balance was positive, the re- uirement was equal to the intake minus the Ca stored. He was naware that only part of the ingested Ca was retained, and nce most of his subjects were in negative balance, the quirement figure he arrived at was too low.

It has now been realized that, unless very carefully con- olled, the results of balance experiments can be misleading. fact, Kraut and Wecker [3] regret there is not some method dependent of metabolic experiments which could be used stead. In the case of children a new method has been sug- sted which will be described below.

Official organizations dealing with research into nutrition ve now published Tables of recommended dietary standards, cluding Ca. The first to attempt this was the League of ations Health Organization, which issued a report in 1935 ]. The chief bodies which have since then recommended andards are the Food and Nutrition Board of the National esearch Council of the U.S.A. [5]; the Committee on utrition of the British Medical Association [6]; the Canadian ouncil on Nutrition [7]; and the Publicity Office of the

Dutch Nutrition Council [8]. The Nutrition Council of So Africa have also recently published a Table of diet standards [9].

The rationale of these various recommendations differs a makes comparison difficult. The resulting limitations ha been well discussed by Pett [10].

The following quoted from the National Research Cou cil Board's report published in 1953 [5] gives a good summa of the positions adopted by the various bodies. With resp to its own figures, the following is stated:

> The recommendations are not intended to provide a ba for judging the nutritional status of population groups b rather to serve as a guide for planning food supplies these groups. If these allowances are used in dietary eval tion, it is essential to appreciate that, while most pers whose consumption equals or exceeds the goal are presu ably adequately nourished, not all persons who fail to rea these goals are malnourished.

They then go on to state:

> The dietary standards proposed by the Committee Nutrition of the British Medical Association are somew similar in philosophy and objective to the Recommend Dietary Allowances (of the National Research Counc Board), but the caloric estimates and quantities of vari nutrients recommended 'are believed to be sufficient establish and maintain a good nutritional state in *repres tative individuals* of population groups'. The British st dard thus differs in providing values which apply to average person rather than values which will cover ess tially all individuals, recognizing, however, that some me bers of every group may need more than the average.
>
> The dietary standard proposed by the Canadian Cou on Nutrition differs considerably in purpose and philoso from the American and British recommendations. Canadian standard represents 'a nutritional floor bene

which maintenance of the health of the people cannot be assumed'. It is designed not only as a basis for planning food supplies for individuals or groups, but also for use in assessing the amount of each nutrient provided by a diet for an individual or group in terms of *probable physiologic requirement*. Recommendations are based upon body size, and the influence of work on requirements is emphasized.

In view of the differences in underlying philosophy and purpose between the Canadian, British and American standards, it is obvious that specific recommendations will differ. Values proposed in the Canadian standard approach minimal requirements; allowances in the British standard are for the maintenance of good nutrition in the average person; allowances in the United States standard are recommended for the maintenance of good nutrition in substantially all normal persons.

The National Nutrition Council of South Africa [9] state in his connexion:

> The standards proposed here should be regarded as adequate for the maintenance of health without allowing for a safety margin for ill health, or for great individual differences in absorption and metabolism.

The recommendations of these bodies for Ca are summarized in Tables I and II.

TABLE I

RECOMMENDED Ca STANDARDS FOR ADULTS

G. per day

| | U.S.A. | B.M.A. | Canada | Dutch | South Africa |
|---|---|---|---|---|---|
| Man | 0·8 | 0·8 | 0·75 | 1·0 | 0·7 |
| Woman | 0·8 | 0·8 | 0·6 | 1·0 | 0·6 |
| Pregnancy | 1·5 (3rd trimester) | 1·5 (last half) | 1·6 (last half) | 2·0 | 1·5 (3rd trimester) |
| Lactation | 2·0 (850 ml. milk per day) | 2·0 | 1·6 | 2·0 | 0·12 more for each 100 ml. milk per day |

## TABLE II

RECOMMENDED Ca STANDARDS FOR CHILDREN
G. per day

*Infants*

| | U.S.A. | B.M.A. | Canada | Dutch | South Africa |
|---|---|---|---|---|---|
| Months | | | | | |
| 1–3 | 0·6 | | — | | |
| 4–9 | 0·8 | 1·0 | — | 0·8 | 0·8 |
| 10–12 | 1·0 | | — | | |
| | | *Children* | | | |
| Years | | | | | |
| 1–3 | | | | | 0·6 |
| 4–6 | 1·0 | 1·0 | 1·0 | 0·8 | 0·7 |
| 7–9 | | | | | 0·8 |
| 10 | | | | | 0·8 |
| 11 | 1·2 | | | | B 0·8, |
| 12 | | B 1·3 | B 1·0, G 1·5 | | G 1·0 |
| 13 | | G 1·5 | | | |
| 14 | | B 1·3, G 1·2 | 1·5 | | |
| 15 | | | | 1·2 | |
| 16 | B 1·4 | | — | | B 1·3 |
| 17 | G 1·3 | B 1·4 | — | | G 1·2 |
| 18 | | G 1·1 | — | | |
| 19 | | | — | | |
| 20 | | | — | | |

### Ca requirements of adults

It will be noted that there is a certain variation mostly
line with the philosophies of the various bodies. The figur
have been arrived at mainly as a result of estimates of the tot
food consumption of the population, information deriv
from surveys, and balance experiments. The B.M.A. com
mittee stated that they 'found no little difficulty in reaching
decision regarding the desirable intake of calcium', large
owing to the ability of the body to adapt to lower intake
The N.R.C. of the U.S.A. had recommended 1·0 g. per d

r adults in their 1948 publication, which the B.M.A. committee thought unjustified, and in the latest Table the standard as lowered to 0·8 g. The question of a 'margin of safety', used originally by Sherman, does not appear to be now considered.

In men and non-pregnant and non-lactating women a figure of 10 mg. per kg. body-weight per day seems to the present writer to be adequate, and the South African figures were based on an average man weighing 73 kg. and woman of 59 kg. There is much evidence to support this point of view. Leitch [11] selected from the literature a large number of balance data on healthy women both in positive and negative balance. On plotting intake against output, the level of Ca intake above which losses and gains of Ca were equal was 550 mg. per day. This worked out at almost 10 mg. per kg. body-weight. Mitchell and Curzon [12] selected 139 observations from the literature on 107 subjects, of whom 18 were men. These results were plotted with respect to intake and output and a straight line fitted to the data. This line intersected the diagonal at 9·75 mg. per kg. body-weight per day, a figure which agrees well with that of Leitch obtained by an analogous method.

In a study of nine normal male subjects [Steggerda and Mitchell, 13], when the availability of milk Ca was being investigated, an average Ca requirement of 9·55 mg. per kg. body-weight per day was arrived at. In 1946 Steggerda and Mitchell [14] did balance experiments on 19 men, the diet periods being 20 days and 75 such periods being considered. In both these studies the subjects were first on a basal diet of very low Ca content (about 200-250 mg. per day); the Ca level was then increased by the addition of various milk products. From these figures they could calculate the percentage utilization of the extra Ca by the method of Kinsman *et al.* [15]. The negative balance was then divided by the percentage utilization and multiplied by 100, which gave the amount of Ca needed to wipe out the lost Ca, and on being added to the intake of Ca gave the total requirement. Thus in a subject with a utilization of 32 per cent., the total intake was 458 mg.

and the balance − 57 mg. This loss would be abolished by t
addition to the diet of $\frac{57}{0{\cdot}32} = 178$ mg.: 178 + 458 = 636 m
which is considered the total daily Ca required. Averaging
the results, the daily Ca requirement came to 9·21 mg. p
kg. body-weight. In a later paper from the same laborato
Bricker *et al.* [16] found, working with 8 women, that t
requirement was 11·8 mg. per kg. body-weight per day.

In their 1946 paper Steggerda and Mitchell [14] summariz
their present and earlier findings, certain unpublished da
and also calculations from the work of Breiter *et al.* [1
who conducted similar balance experiments. The mean of t
daily Ca requirement per kilogram of body-weight vari
from 9·21 to 11·61 mg., and averaged for 43 individua
9·99 mg. Taken in conjunction with the suggestions, usi
different methods, by Leitch of 10·0 mg., and Mitchell a
Curzon of 9·75 mg., it must be agreed that the correspondenc
is quite remarkable. In view of the findings of Steggerda a
Mitchell, Walker *et al.* [18] adjusted the Ca intake of three
their subjects to a level of 10 mg. per kg. per day and fou
that they either were in equilibrium on this intake or e
adapted to it without difficulty.

Steggerda and Mitchell [14] recommend a daily intake
10 mg. per kg. body-weight per day, provided that an avera
proportion of Ca comes from dairy products. They do n
feel that a 'margin of safety' is needed. In the case of the in
vidual who has been accustomed to more, the adapti
mechanism will come into effect and, judging from t
experiments quoted elsewhere, will cause no physiological d
turbance at this level of intake.

The figures of the N.R.C. of the U.S.A., and the B.M.A
and those for Canada and South Africa are fairly closely
accord with the information available in the literature, b
the present writer feels that the Dutch recommendations a
unnecessarily high. No harm of course comes from this high
consumption of Ca, but from an economic point of vie
when the supplies may be limited, and especially when the

a great deficiency in other parts of the world, unnecessarily igh standards should be avoided. It should be recalled that legsted and his colleagues [19] found perfectly healthy people n equilibrium on intakes of 100 and 200 mg. per day, and tated, as has been emphasized above, that all considerations f Ca requirements are really studies of the previous Ca in- ake, and that Ca deficiency is unlikely in adults (except omen in the child-bearing age) on most natural diets. Thus he reservation must be made that the recommended allow- nces of Ca are those for people accustomed to eating Ca at hat level. If our dietary habits changed, it is possible that the Ca requirement would too.

*Pregnancy.* As has been stated above, pregnancy does not nvolve a great strain on the Ca metabolism of a woman pre- iously adequately nourished in this regard and it is easy, knowing the Ca content of the foetus, to calculate the extra requirement.

According to figures published by Mitchell and Curzon [12], Ca accretion on any scale does not begin till the 7th month and most standards are applied only to the last half of preg- nancy or to the last trimester. The Ca content of the foetus at term is usually about 25 g., the other products of conception being virtually Ca-free. If extra Ca is given over the last trimester (of 93 days) 270 mg. per day must be available to the foetus. Presupposing utilizations of 25 or 30 per cent., 1,000 or 800 mg. respectively must be added to the maternal diet. The figures recommended by Canada, Holland and South Africa allow extra Ca at these levels, but those for the U.S.A. and Great Britain are less. It must be admitted that the utili- zation of the extra Ca is not accurately known, and it is possible that it is more efficient in pregnancy. With a total body Ca of over a kilogram, it might be thought that the loss of a few grams would be immaterial. But with the strain of lactation coming soon, no drain of the body Ca stores should be allowed at this time.

*Lactation.* As with pregnancy, it should not be difficult to arrive at figures for the Ca supply to the mother's milk, which

on an average contains 0·03 per cent. The U.S.A. figures a
1·2 g. extra Ca, supposing that 850 ml. of milk are produc
daily. This milk would contain 255 mg. Ca and thus abo
20 per cent. utilization of the extra Ca has been allowed f
The B.M.A., Canadian and Dutch figures allow 1 to 1·2
extra Ca per day and the South African tables state th
0·12 g. extra Ca should be taken for each 100 ml. of m
produced, the utilization being placed at 25 per cent. Wh
these figures are mathematically correct, it is not infrequen
found during lactation that bone Ca is used in preference
that from the food. This difficulty cannot be overcome, a
the best is to give the mother the extra Ca she should get, so
or probably a great deal of which she will use in lactatio
and with a proper diet after weaning any losses will soon
made good. If she had no extra Ca at all and produc
1,000 ml. of milk per day for 9 months (a peak output), s
would lose altogether about 80 g. of Ca or about 8 per ce
of her stores.

**Ca requirements of children**

*Infants.* This is probably the most difficult of all estima
to arrive at, mainly because the degree of utilization of the
of milk is not accurately known. It might be expected th
some idea could be got from the consumption of mothe
milk, a daily intake of 1,000 ml., which is a top figure, givi
300 mg., which presumably is about the maximum a ba
needs till it is weaned.

Turning, on the other hand, to balance experiments and
investigations such as those of Holmes [20], one finds that t
calculated daily Ca retention of a baby in the first month
as high as 600 mg. There is some discrepancy here, since ev
if the baby's retention was 100 per cent. [and it is probably
more than 60 per cent., Telfer, 21] it could not obtain this
take without imbibing 2 litres of human milk, and more if t
retention was less. Even at 6 months the breast-fed ba
would not be getting enough Ca. Most of the recommend
tions for Ca for infants in Table II pertain to cow's milk, p

imably on the supposition that the retention of breast milk a is much higher than that from cow's milk. Even so, these gures are not very realistic. To get 1 g. of Ca, a baby would ave to drink over 800 ml. of unmodified cow's milk. In a udy of Ca requirements by the present writer published in 950 [22] he stated that he did 'not feel competent, in the ate of present knowledge . . . to recommend any figures for he first period of life'. His feeling is unchanged. Only accurate alance work can solve this problem.

*Children.* The figures in Table II show a general agreement hat the Ca intake should be about 0·8 to 1·0 g. per day in xtreme youth, rising to 1·2 to 1·5 g. during the period of dolescent growth. While these recommendations are undoubtedly adequate, some of them have a 'penny in the slot' ook which would indicate that not a great deal of thought had been given to them as average or maintenance allowances. Thus it is hardly possible that the Ca requirement, which is to supply Ca for growth, would be the same between the ages of 1 and 11, or between 10 and 20.

To study the matter more objectively, the present writer [22] made calculations for boys based on the calculated Ca ncrements published by Mitchell *et al.* [23]. This method was one proposed by Terroine [24], who used it for determining he nitrogen requirements during growth. The information necessary is the daily Ca accretion in the body, and the degree of retention of the dietary Ca. The latter figure is the hardest o arrive at, and was placed arbitrarily at 25 per cent. for -2 years and 20 per cent. for the rest of the growth period. As pointed out earlier, the retained Ca is all used for growth nd calcification processes, and no maintenance requirement as to be considered. The fraction

$$\frac{\text{Daily Ca accretion} \times 100}{\text{Percentage retention}}$$

ave the daily requirement. The figures arrived at were far ower than those recommended by any official body, being 0·65 g. for 1–2 years, 0·4 g. up to 7 years, then gradually rising

to a top figure of 1·3 g. for ages 14 and 15, after which th allowance gradually fell to the adult one of 0·7 g. when maintenance requirement appeared. The present writer sti feels that these figures are a better representation of the C needs during growth. The South African Nutrition Counc followed in general the arguments used by Irving, and thei recommendations are mostly lower than those of the othe bodies. When reliable figures, based on analyses of norma children, exist, this method will be the natural way of deter mining the Ca needed during growth.

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## CHAPTER IX

# The Calcium of the Blood

THE Ca content of blood in man is remarkably constant und physiological conditions and over all ages. It is virtually present in the plasma (or serum, where it is usually estimate at a level of 9 to 11 mg. per 100 ml. This constancy is se in many other animals, but in the rabbit the plasma level subject to great variations, rising as high as 19 mg. und normal conditions. The blood-cell of the ox, sheep and rabb unlike those of man and pig, contain variable amounts of C

Experimental raising of the blood Ca to high figures fatal. When the Ca falls in amount, the condition tetany sup venes when the level has reached about 6 mg. per 100 m This is characterized by typical muscle spasms, usually att buted to increased sensitivity of the neuro-muscular junctio though Greenberg *et al.* [1] think that a part of the centr nervous system higher than the spinal cord is involved.

The Ca in the plasma is present in two forms, ionized a diffusible, and non-diffusible. Benjamin and Hess [2] su gested that the Ca was present in four fractions, but this co cept has not been generally accepted.*

The determination of the proportions of ionized and no diffusible Ca presents some difficulties; if simple dialysis carried out, all the Ca will be removed from serum, since t two fractions are in equilibrium. A procedure known as co pensation dialysis can be used instead, in which serum dialysed against a number of solutions of different Ca co tent. The solution in which no change of Ca concentrati occurs contains Ca ions at the same level as the serum. Wi this method, about 60 per cent. of the Ca of human serum

* There is a very small diffusible fraction of unionized complexes in plasma.

und to be diffusible. Greene and Power [3], using the ethod of vividiffusion, in which the entire blood of a living imal is dialysed against a small volume of fluid, reported on average 62 per cent. of the Ca to be diffusible. Ultra-tration under pressure through a collodion membrane gives ures of much the same order [Greenberg and Larson, 4]. cLean and Hastings [5] made use of the sensitivity of the g heart to Ca ions and found about half the total serum a to be diffusible and nearly all of this in an ionized state. alculations based on the composition of cerebrospinal fluid, edema fluid, ascites or pleural effusions indicate that the ffusible Ca of normal serum is about 5 mg. per 100 ml. thus appears fair to conclude from all these methods that is fraction lies between 5 and 7 mg. per 100 ml.

Turning to the nature of the non-diffusible fraction, there ems hardly any need to consider the older views, since all vidence now points to this being a Ca-protein complex. cLean and Hastings [5] showed beyond doubt that a rela-onship exists between the Ca ions and the protein of plasma nd have calculated mass-action equations and an ionization onstant. They have constructed a nomogram from which, nowing the values of total protein and total Ca, the Ca ion oncentration can be derived. The mass-action considerations volved have been shown to be true, as regards the proportion f ionized and unionized Ca, over a wide range of values as in ypo- and hyperparathyroidism, provided the protein content f the plasma does not change.

The ability of blood protein to bind Ca has been demon-rated by a number of workers. McLean and Hastings [6] rst attempted a quantitative study with purified albumins repared from the sera of horses and oxen. Martin and erkins [7] found the serum albumin of horse and ox to bind bout 11 mg. per g.N. and in humans the corresponding figure as 4·7 to 4·8 mg. Repeated recrystallization could alter this inding power. In a subsequent paper [8] the binding power f human albumin was found to be very constant at 4·7 mg. 0·9 mg. per g.N. over ages from 2 to 91, and the same in

both sexes. In hyperparathyroidism the combining power increased. Carr [9] reported that bovine albumin took u maximum number of 8 Ca ions per molecule of protein pH of 7. With increased pH the binding power rose, and with decreased pH. The binding of Ca in this way mus of a loose type, since as stated above, all the Ca can dialysed out of serum. Armstrong *et al.* [10] investigated *in vivo* using $Ca^{45}$, and reported that the protein binding not impede the transcapillary movement of Ca and that redistribution of Ca between the ionized and bound fo was rapid. The transcapillary movement of $Ca^{45}$ and I were the same.

Hopkins *et al.* [11] studied this problem in a different They added Ca to normal serum and found that as the con was increased up to 19 mg. per 100 ml., the ionized and bo Ca fractions rose together, in an extraordinarily fixed portion, the serum having the capacity to 'carry' more than it normally did. The P up to this point was unchan When a figure of 20 mg. per 100 ml. was reached, the centrations of filtrable Ca and of P fell, possibly due to formation of a supersaturated solution. An important p made by Howard [12] was that there was no reciprocal tionship between Ca and P concentrations in the serum *in* and thus any such changes seen *in vivo* were the resu physiological mechanisms. This has been shown by Mc and Hinrichs, and Gersh [13], who found that the injec of phosphates caused the formation of a colloidal Ca p phate complex in the blood, which was rapidly remove the liver and spleen.

Another aspect of the matter has been studied, viz. blood-changes in hens during the egg-laying period. At time both the total [14] and non-diffusible Ca in the pla rise [15]. Clegg and Hein [16] found that after giving die stilboestrol the rise in non-diffusible Ca in chick serum paralleled by a rise in concentration of two of the ele phoretic components, and in a later paper [17], after sim treatment found a 'new' protein which was rich in P

identified with $P^{32}$. When the Ca in the serum was raised, the electrophoretic mobility of this protein was markedly reduced, but that of the others was not, and this was attributed to combination with Ca.

*Factors affecting the blood Ca level.* Starvation usually has little effect upon this. But Ca deficiency will in the end cause the blood Ca to fall. Greenberg and Miller [18] found that a Ca-deficient diet fed to growing rats caused a fall in the blood figure from an original level of 10–12·5 mg. per 100 ml. to one of 7 mg. after 40 days and to 6·1 at 68 days. By this time the body Ca stores had fallen to less than half and the authors considered that a significant reduction in the Ca stores must take place before the blood Ca is reduced. Interestingly enough, tetany was not seen, nor did Irving [19] observe it in rats on low Ca diets and with serum Ca values of 5 mg. The injection of NaF, which was carried out for another purpose, into such rats immediately precipitated violent tetanic seizures. Presumably the ionized Ca is not lowered to the same extent as the total Ca.

In animals with well-marked low P rickets, as caused by the Steenbock-Black diet, the blood Ca level is usually a high normal and the blood inorganic P greatly lowered. When such animals are starved, the blood inorganic P rises rapidly, due to mobilization of P from soft tissues, and the Ca simultaneously falls at a fast rate, the animals looking miserable and being liable to tetany [20]. These changes are presumably due to the formation of a colloidal Ca phosphate complex and its removal from the blood as described above. When animals on a low Ca diet are starved, the blood Ca remains at a constant low level for as long as 10 days [19], and is presumably kept depressed by the continual mobilization of phosphate. A rapid and fatal hypocalcaemia can be induced by the injection of ethylene diamine tetracetic acid (versene) [Popovici *et al.*, 21].

*Vitamins.* The only vitamin with profound effects upon the blood Ca is vitamin D and associated compounds. McLean [22] held that there were two actions of vitamin D, antirachitic

and calcaemic. The antirachitic effect is self-explanatory and caused by a low-dosage level, capable of curing rickets. A cording to the older observations of Kramer *et al.* [20], t Ca × P content of the blood had to rise, under the action vitamin D or other curative agents, to a certain height befo calcification of rachitic cartilage occurred. The rationale this concept, which though empirical has been most helpf is explained elsewhere (see p. 117). This antirachitic dose le of vitamin D has no effect upon the blood Ca level of norm animals or humans, but raises the blood P in animals sufferi from low P rickets, and the Ca in those with low Ca ricke largely due to its action on intestinal absorption and possi also to its effects upon renal function. Vitamins $D_2$ and are equally effective in this respect in mammals.

The calcaemic effect of vitamin D has been used chiefly the treatment of tetany and very large doses of the vitam are used. Here the effect is caused by the destructive acti upon bone [McLean, 22]. Other substances allied to vitamin have been used, especially dihydrotachysterol or A.T. McChesney and Messer [23] found, using massive doses giv to dogs, that $D_3$ caused a longer lasting hypercalcaemia th $D_2$, taking up to 26 days to subside, and A.T.10 produced quick rise and fall in blood Ca. Parathyroid extract has a ve quick action, the hypercalcaemia being over in 36 hou [Collip and Clark, 24]. McLean considers that the effect A.T.10 is, like that of vitamin D in large doses, directly up bone. In addition it has marked effects upon kidney functi decreasing P reabsorption in the tubules [Harrison and Har son, 25] and thus secondarily affecting bone, mobilizing bo salts and thus raising the blood Ca. A.T.10 can probab increase intestinal Ca absorption in the same way that vitam D does, but Albright *et al.* [26] consider that any antirachi action is largely overshadowed by its marked action on excretion. In contrast, parathyroid extract, which also has calcaemic effect, has no action on intestinal Ca absorpti and is therefore not antirachitic. Its effects elsewhere will considered below.

It might be mentioned at this stage that suggestions have been made that vitamin D acts through the parathyroid glands. This was first suggested by Taylor *et al.* [27], who claimed that the toxic actions of vitamin D were not obtained after parathyroidectomy. This was not confirmed by Dale *et al.* [28]. Tweedy *et al.* [29] found it was difficult to raise the low blood Ca level after parathyroidectomy with vitamin D; but if the animals were put on to a high Ca : P ratio diet the blood Ca became higher and it was then possible to demonstrate the calcaemic action of the vitamin. Thus it appears there is no connexion between the effects of vitamin D and the parathyroid, though their actions are in some instances similar.

*The endocrine glands.* Some of these have profound effects upon the blood Ca level, but others have but little action. Thus insulin very probably increases the blood Ca, but secondarily to its effect of lowering the inorganic P level. The thyroid has a considerable effect upon Ca excretion and in thyrotoxicosis there is an increased loss of Ca. There seems to be no unanimity about the level of blood Ca in this condition. Albright *et al.* [30] considered it to be slightly raised, but Wade [31] found it to be lowered. Robertson [32] reported that it was lowered from an average figure of 10·39 to 9·71 mg. per 100 ml., the difference proving to be statistically significant. In myxoedema the level is unchanged.

*Oestrogens.* As stated above, the blood level is raised in birds during the egg-laying cycle, and in non-ovulating birds by the administration of oestradiol or diethylstilboestrol, this being accompanied by the formation of medullary bone (see p. 106). This was still observed in birds after parathyroidectomy or hypophysectomy, so that the action of oestrogens was not through these glands [Riddle and McDonald, 33].

*Pituitary.* The reports in the literature are somewhat conflicting. Anderson and Oastler [34] in well-controlled experiments with rats found that hypophysectomy had no effect upon the blood Ca level, and assumed that the existence of a parathyrotropic hormone in the pituitary was not proved, which is still the consensus of opinion. Since the effect of the

anterior lobe of the pituitary on bone is directed mo matrix formation than calcification, it might be expected the blood Ca level would not be affected.

*Adrenal glands.* Cortisone causes a greatly increased e tion of Ca in the urine and faeces, but in otherwise no animals both cortisone and adrenocorticotropic horm cause little change in the blood Ca or, if anything, a [Pincus *et al.*, 35]. If, however, the animal is nephrectom cortisone causes an increase of serum Ca to toxic l [Grollman, 36]. Adrenalin administration is followed rise in serum Ca [Pincus *et al.*, 35].

*Parathyroid glands.* These glands have an important trolling influence upon Ca metabolism. They are sensitiv the blood Ca level, since there is an inverse relationshi tween this level and the volume of the gland [Stoerk Carnes, 37]. An active extract was prepared in 1925, w could increase the blood Ca level and produce all the ef seen in hyperparathyroidism. The exact mode of action o hormone is still undecided, there being two schools of thou one that the effect is directly upon bone, the other tha action is on the kidney, bone being secondarily affected.

The original view was that the hormone acted directly u bone, but it was soon challenged by Albright and Ellsw [38], who considered that this effect was secondary to excretory changes, the tubular reabsorption of P b decreased, leading to increased P excretion. Since then evidence has been produced supporting this concept. Har and Harrison [39] reported parathyroid hormone to dec tubular P reabsorption, and Tweedy *et al.* [40] in parti found the urinary excretion of $P^{32}$ in parathyroidecto animals to be most sensitive to small doses of parath extract and judged it must act directly on the kidney. D and Gordon [41] likewise found that the serum P of thyroidectomized rats could be easily reduced by dose which hardly affected normal animals.

Talmage and his colleagues have done a conside amount of work, and seem to subscribe to both views. S

ng the metabolism of both $Ca^{45}$ and $P^{32}$ they considered that he hormone acted primarily on bone, this effect being enanced by an additional action on the kidney, whereby the excretion of P was increased [42]. They pointed out that parathyroid extract raised the blood P, even though the renal excretion of P was ten times the normal level, so that P was entering the blood faster than it could be removed and presumably must have come from bone.

In a subsequent paper [43] they stated that the renal excreion of P was markedly reduced one hour after parathyroidecomy and considered that one action of the gland was to control renal excretion of P, the renal thresholds being altered n such a way that that for P was increased, while that for Ca was diminished. The parathyroid kept the blood Ca at 11 mg. per 100 ml. serum, but in its absence the figure fell to 6–7 mg. 44]. They seem to feel that while renal phosphate excretion is not the primary means by which the parathyroids influence Ca and P metabolism, none the less in the rat they do directly modify the excretion of both elements.

The pure renal theory has lost a good deal of ground, and even Albright and his colleagues now agree that this is not the only action of the hormone [45], since they found evidence of osteoclastic resorption in bone of nephrectomized animals njected with parathyroid extract. Stoerk [46] reported that the parathyroid hormone could still cause hypercalcaemia and even osteitis fibrosa after nephrectomy. Monahan and Freeman [47] stated that the serum Ca was still depressed in parahyroidectomized dogs after nephrectomy. Other workers have produced similar evidence that parathyroid hypercalcaemia can still be obtained after nephrectomy [e.g. Grollman, 48] or that, under appropriate conditions, the renal effect on P xcretion is absent [Hogben and Bollman, 49] or much lessned if the urinary P was reduced [Coxon, 50]. Munson [51] n a recent paper stated that the fall in serum Ca after parahyroidectomy was not dependent upon a prior rise in serum norganic P and that injection of the hormone into parahyroidectomized rats raised both the serum Ca and P. He

concluded there was a direct action of the parathyroid up skeletal tissue independent of whatever effect it might have the kidney tubule. Both Barnicot [52] and Chang [53] ha reported that when fragments of bone and of parathyr gland are grafted together subperiosteally or intracerebral bone resorption with an osteoclastic response occurs, the eff being specific and not caused by other glands and tissues teste

Munson raised the interesting suggestion, for which there increasing evidence, that there are two hormones in parath roid extract, Ca mobilizing and phosphaturic. Handler *et* [54] found different effects if the hormone was given su cutaneously or intravenously, and Handler and Cohn [5 suggested from their findings that there were both hyp calcaemic and haemodynamic factors, the latter increasi the renal plasma flow and glomerular filtration rate, t factor not being essential for hypercalcaemia. Stewart a Bowen [56], using the Tepperman technique for the prepa tion of parathyroid extract [57], got a substance from t thymus and spleen which stimulated P excretion by the kidn and considered the phosphaturic effect of parathyroid extra to be an artefact resulting from the extraction process, sin formalin treatment caused a loss of the hypercalcaemic pow but not of the renal factor. Davies and Gordon [41] found t factors in parathyroid extract, a dialysable one affecting t renal P excretion, and a non-dialysable one which affect both urinary P and also the serum Ca; more of the latter fra tion had to be given to obtain the serum Ca action. Ken *et al.* [58] reported that there were discrepancies in the ra of hypercalcaemic to phosphaturic activities of differe parathyroid preparations.

It thus seems clear from this, that the disagreement in t literature on the mechanism of parathyroid action may w be due to the existence of two factors and the different prop tions of these in different preparations. None the less, t present writer feels, on the balance of the evidence, that t primary action of the gland is on bone, and the bone chang are not secondary to alterations in renal P excretion.

Parathyroidectomized animals will develop rickets like unoperated controls and their cartilage will calcify *in vitro* [Heimer *et al.*, 59] and the evolution of hypervitaminosis A in rats is unaffected by parathyroidectomy [Cohen *et al.*, 60]. As stated above, parathyroidectomy does not prevent the hypercalcaemia caused by oestrogens [33].

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## CHAPTER X

# Bone Formation and the Influence of Various Factors upon this

A SECTION on histology may seem somewhat out of place in a monograph primarily biochemical. But there is a close correlation between the structure of bone, especially during development, and its chemical changes, and a brief account of the microscopic appearances must be given. Furthermore, certain parts of the bone, especially the rachitic cartilage, have been used in enzyme studies. The reader who wants a more complete description is referred to such works as that of Weinmann and Sicher [1].

There are two types of osteogenesis—endochondral and intramembranous. In both, specialized cells are concerned with bone formation and removal. The former cells, osteoblasts, are differentiated mesenchyme cells; they can be seen to take on this function especially in the early stages of intramembranous bone formation. They are usually cubical or flattened cells, with cytoplasm staining strongly with basic dyes, and lie in a row along the face of the bone they are forming. The 'bone removing' cells, or osteoclasts, are still one of the mysteries of osteogenesis. They are large cells with many nuclei, usually about six, and the cytoplasm takes up acidic stains in a characteristic way. They also lie along the bone face and often in little niches called Howship's lacunae. In spite of dogmatic statements by various authors, the present writer does not believe that the functions of these cells is yet settled. They are always present where bone resorption is occurring and they can appear and disappear with considerable

speed. No mitoses are seen in their development, a
has been suggested that they may form by fusion of o
blasts. Compared with osteoblasts which line the bone fa
a continuous row, osteoclasts are seen less frequently but
have been shown to be motile [Hancox, 2]. Whether
actively resorb bone is not proved, but it is possible that
ingest the products of bone resorption since Bailie
Irving [3] found silver staining granules in them. They are
macrophages in the accepted sense as they will not tak
dyes like trypan blue. They appear to have a fibrillary ma
next to the bone that they touch which some workers
associated with bone resorption, but Ham [4] has sugge
that this is due to the fibrils of bone becoming visible du
resorption. An excellent review of all the possibilities has
written by Hancox [5]. Heller *et al.* [6] studied the bone
lular changes in hyperparathyroidism, from which it app
that a kind of dynamic equilibrium exists between the
associated with bone formation and destruction, so
changes from one to another can take place with ease. W
this concept is a great advance, it still does not explain
detailed influences causing changes in this equilibrium nor
particular parts of a given bone are handled in a particular

In resorption of bone, removal of matrix and the calc
elements proceed at the same time. Removal of lime s
leaving behind the uncalcified matrix, a process called h
teresis, is not considered to occur [McLean and Bloom, 7

In intramembranous bone formation, certain of the me
chyme cells take on the functions of osteoblasts. In the g
ing animal, one finds the appositional side of the bone l
with osteoblasts and the resorptive side with osteoclasts
the osteoblasts lay down bone, they become enclosed in
osteocytes or bone cells, with long processes extending thro
the matrix. Whether the osteoblasts merely calcify prefor
matrix or actually make it as well is not known, but thick
lagenous fibres can usually be seen extending from the ti
round the bone, between the osteoblasts and into the b
substance [Irving, 8], rather like coarse Korff fibres associ

ith dentin formation. Uncalcified bone matrix is called os-oid and normally this is rarely seen since matrix formation nd calcification proceed at equal speeds. Only when bone ormation is very rapid does one see uncalcified margins of steoid. Calcified matrix differs from osteoid in its histological aining, being strongly basiphilic while osteoid is eosino-hilic. Osteoid margins are seen typically in rickets when cal-ification lags behind matrix formation, and even after histo-gical decalcification the difference in staining is still present. Veinmann and Sicher [1] suggest that some chemical change ccurs in the organic cementing substance of osteoid before can be calcified, and that this causes the difference in aining reaction.

Endochondral ossification is seen usually at the ends of ng bones. Here a cartilage matrix is first used as a scaffold r the formation of the primary spongiosa, which is then re-odelled in accordance with the stresses and strains imposed n the bone. Concurrently the shaft increases in width and ickness by processes of intramembranous ossification.

Dodds and Cameron [9] have given a very complete sum-ary of the normal process and that occurring during rickets. hey divide the epiphyseal cartilage into five zones: 1, reserve lls, a narrow layer; cells divide to produce daughter cells. cell multiplication, a wide zone of up to 12 cells in thickness; lls divide to produce groups of 4 cells. 3, cell growth, the lls becoming square in profile; not a wide zone. 4, fully own or hypertrophic cells; these cells look almost like plant lls, being large and translucent in appearance; they contain uch glycogen; calcification starts in the longitudinal walls etween the cell columns. 5, cartilage removal; by this time e cartilage cells are considered by many to be dead; narrow ngues of marrow, rich in capillaries, advance and remove e transverse cell walls and also some of the longitudinal alls between the cell columns. The remaining longitudinal alls are used as a foundation for bone deposition (primary lcification). Calcification is usually 3–4 cartilage cells ahead f cartilage removal.

A striking aspect of the whole picture is the regularity direction of the cartilage cells, which lie in columns of s lines from where they are first formed, through the v phases of their development, until they are finally erod removed.

At the site of primary calcification a honeycomb appe is seen of calcification on the remaining cartilage (p spongiosa). As one progresses down the diaphysis, co able remodelling occurs, stouter trabeculae of pure bo place the more slender ones, and these new trabeculae, b sideways, make a firm connection with the shaft. Frag of cartilage may still be seen in quite mature trabecula may persist for a long time. Below this secondary spo most long bones form a tube which is filled with marr

The spongiosa forms a readily available store of Ca can be easily withdrawn. This was shown by Bauer *et a* using alizarin red which stains the most recently formed Warnock and Duckworth [11] have also found the sam

With the cessation of growth, the epiphysis closes a cartilage disappears. The formation of bone and excha bone salts do not stop however with the cessation of g Bone is a most labile tissue, and in spite of its apparent of structure, remodelling and replacement go on continu The osteoporosis of old age is due to breakdown being q than new formation.

## EFFECT OF VARIOUS FACTORS UPON OSSIFICATI

### Dietary

*Ca, P and vitamin D.* Deficiency in any or all of these histologically very similar appearances, those of ri Experimentally it is possible to produce rickets in gr rats and rodents in general using diets with a Ca : P ra 4 or more (e.g. the Steenbock–Black diet), and a simil not so marked condition is seen if the dietary Ca : P r low, around 0·25. The requirement of vitamin D in rats i low [Irving, 12] and vitamin D free diets cause only

nterference with calcification. Rickets is easily produced in puppies with diets low in vitamin D [Mellanby, 13] and in hildren vitamin D deficiency is the usual cause, though cases of rickets reported in South Africa, where considerable solar adiation should ensure adequate vitamin D, may be due to Ca deficiency.

The prime change in endochondral ossification in rickets s an inability to calcify and erode the hypertrophic cartilage ells. There is some disagreement over which of these processes stops first. Dodds and Cameron [9] consider failure of alcification to be the prime fault and Park also holds this iew [14]. Shohl and Wolbach [15] consider that the cartilage ells do not degenerate and thus capillary invasion, which hey regard as comparable to granulation tissue formation following tissue defect, does not occur. In the early stages calcication and cartilage removal are at the same level instead of eing 3–4 cells apart. Later, calcification of the hypertrophic ells ceases completely and with it cartilage removal. The ypertrophic cartilage cell layer becomes greatly thickened nd forms a new part of the bone structure called the metahysis, which is visible radiologically as a widened transucent zone. Osteoid is often deposited on this cartilage. Later n the condition a lawless invasion and removal of cartilage n all directions, called 'resumed cartilage removal' by Dodds nd Cameron, occurs, the eroded spaces being filled with wisted masses of osteoid. The rachitic metaphysis has been sed by those studying the enzymatic changes in calcification, s it will recalcify *in vitro* if placed in appropriate media.

The changes in intramembranous bone during the onset of ickets have been recently described by Bailie and Irving [3]. he first change is a slowing of resorption, the bone gradually ecoming wider. Ten days after being placed on the rachitoenic diet, an osteoid margin appears on the appositional side f the bone. This margin gradually becomes wider until by wenty-eight days the bone is entirely osteoid. Between the 0th and 28th day the osteoblast, while retaining the ability o make matrix, gradually loses the power to calcify it.

Resorption of the uncalcified matrix does not occu
the osteoclasts gradually disappear.

As a result of the large amount of osteoid which r
formed, the proportion of calcified mass to total mass
bone is changed. This is usually estimated as the bo
which may fall from the normal value of about 65 pe
of dry fat-free bone to less than half this figure. In the v
opinion the bone ash figure alone is misleading in the
ment of rickets, since he regards rickets by definitio
histological change caused by biochemical factors.

Rickets can be caused by other factors precipitating
phates in the gut, e.g. iron [16], strontium [17] and be
[18]. Be and Sr have in addition a direct action on calcif
processes in bone.

Osteomalacia is a form of adult rickets. Since endoch
ossification has ceased, no epiphyseal changes are see
the process of calcification of replaced bone is interfere
and osteoid is laid down instead. This condition is rare
in Europe, but cases have been described in the Orient
cially in women after multiple pregnancies and lact
when the skeleton has collapsed and crippled the p
Osteoporosis is a different condition and has a definite
ing [Albright, 19], implying a deficient formation of
which is normally calcified.

Rickets can be healed by giving vitamin D, starvatio
a high Ca : P ratio diet is used (owing to mobilizatio
from the soft tissues), or by adjusting the Ca : P ratio
diet. With vitamin D the classical 'line' response is seen
early stages, due to the deposition of lime salts in a line
the metaphysis, where they can be visualized with silver
Six stages of healing have been described by Dyer [2
Coward [21] and they form the basis of the biological e
tion of the vitamin. If the animal is given extra Ca o
the diet, healing is usually so precipitous that no line i
the whole metaphysis filling with calcification. The site
line and, in fact, the development of it depends, in the
writer's experience, on the development of a high deg

ickets and a great deal of 'resumed cartilage removal', since alcification occurs at the edge of the epiphyseal cartilage, owever wide, and if the metaphysis is all cartilage the new alcification occurs at the junction with the diaphysis and no ne is seen. It is sometimes stated that the new calcification ccurs where it would have done normally. This is true in he sense that the edge of the epiphyseal cartilage is always the ite of calcification, but geographically this can be anywhere n the metaphysis depending on the degree of 'resumed car-ilage removal'. Exactly the same kind of calcification is seen *n vitro* if the end of the rachitic bone is incubated in a calcify-ng medium.

The action of vitamin D upon intestinal absorption has been escribed in an earlier chapter. There is a good deal of evi-ence that the vitamin has other actions. McGowan [22] some ears ago suggested that it mobilized P inside the body, pos-ibly from phospholipins. Irving [12] found in a study of the ental reactions to the vitamin that with small doses as much s eight days might elapse before new calcification began and onsidered this must be due to the time taken to mobilize rom inside the body the elements needed for calcification, nd more recently Underwood *et al.* [23] have reported that itamin D mobilized P from organic sources.

Work with radio-Ca shows that vitamin D acts directly on, t any rate, rachitic bone. Greenberg [24] gave rachitic rats $Ca^{45}$ by injection combined with vitamin D by mouth, the ontrols getting no vitamin. The percentage of the dose enter-ng the skeleton in the controls was 28 per cent., and in those etting vitamin D, 45 per cent. Migicovsky and Emslie [25] ould not confirm this in chicks and considered there was no irect action of vitamin D on bone; vitamin D had the same ction in rats and chicks, but there was a difference in Ca etabolism and especially in the effect of vitamin D on the enal excretion of Ca and phosphate, the take-up of Ca by he rat bones being secondary to this [26]. However, Under-ood *et al.* [23], in their turn, did not agree with Migicovsky nd his colleagues and produced strong evidence for their

objection. When $Ca^{45}$ but no vitamin D was given to or rachitic animals, there was a rise in blood Ca and bone $Ca^{45}$ due to exchange. When vitamin D was give was a later second rise in bone $Ca^{45}$ (especially in the animals) but not in the untreated animals, at a time w blood Ca was not rising, or was even falling. Wo radio-P has shown exactly the same [Morgareidge an ley, 27], the bone $P^{32}$ rising only in rachitic animals vitamin D, while the blood $P^{32}$ level was the same these and control animals. Claasen and Wöstman ported similar findings. Cohn and Greenberg [29] fou inorganic P of rachitic rat bone to be raised by vita and they postulated that the vitamin facilitated the sion of organic to inorganic P. Thus the evidence is that vitamin D has a direct action on bone, as well effects upon intestinal absorption.

An interesting action of vitamin D upon the interm metabolism of bone has been recently described by and Patwardhan [30]. These writers found that cartila untreated rachitic rats could not oxidize pyruvic acid, b the cartilage from rachitic rats given vitamin D could The oxidation of pyruvic acid by liver and kidney homo of rachitic rats was unimpaired.

The effects of excessive amounts of vitamin D on bon been studied by Ham and Lewis [31]. The chief chang overgrowth of matrix and the development of osteoid Cortical bone is absorbed to a great extent and covere large amounts of osteoid, the process being unaccom by an osteoclast response. Barnicot [32], using his im tion technique, also found that pieces of bone attac small pellets of calciferol and grafted intracerebrally int mate mice, underwent diffuse resorption.

*Vitamin A.* In deficiency of this vitamin, a curious growth of bone takes place. This was first described by *et al.* [33] in calves and it was shown to be prevent $\beta$ carotene [Moore, 34]. Wolbach [35] considered that th mal processes of bone remodelling were accentuated, b

seems improbable since new bone development can take place on sites where normally resorption occurs. Mellanby [36] also analysed the changes and showed that the position of osteoblasts and osteoclasts could become reversed on certain bones, and this accounted for the development of new bone in abnormal situations. Irving [37] considered that the action of osteoblasts became uncontrolled in the absence of vitamin A so that they formed excessive bone of a primitive type. In areas where normally resorption occurred, but bone was laid down in avitaminosis A, the adjacent cells actively divided and became osteoblasts [Irving, 8]. In hypervitaminosis A, bone formation ceases and fractures occur [Moore and Wang, 38], possibly due to suppression of osteoblast activity, resorption continuing in a normal way [Irving, 37]. Barnicot [39] found that when bone implanted into the brain was in contact with a crystal of vitamin A, resorption occurred with many osteoclasts (this action being specific to vitamin A) and proposed that the action of the vitamin in excess was to cause an active resorption. Fell and Mellanby [40] using mouse foetal bones in tissue culture found that vitamin A in excess caused resorption of the bone but no great number of osteoclasts were present. In long bones which fracture, no particular cellular reaction is seen and it is possible that the fault lies in the organic matrix.

*Vitamins of the B group.* The changes in bone formation during deficiency of riboflavin, pantothenic acid and pyridoxine have been investigated. In riboflavin deficiency [Nelson *et al.*, 41] a gradual cessation of calcification occurs, the primary spongiosa disappearing, the epiphyseal cartilage becoming narrower and finally being sealed off with bone. Virtually the same was seen in pantothenic acid deficiency [Nelson *et al.*, 42]. Silberberg and Levy [43] reported similar findings in pyridoxine deficiency. This picture is presumably more due to interference with matrix formation than to diminution of calcification, and is not apparently specific to lack of these vitamins, as an identical picture is seen after inanition, protein deficiency or hypophysectomy [44].

*Ascorbic acid.* In scurvy a very characteristic picture en due to interference with the activity of the osteoblast, modulates back to a fibroblast-like cell. A great deal of has been done on this condition. Delf and Tozer [45] pub an early account of experimental scurvy, and Wolbac Howe [46] described the changes in great detail, as ha been done by many writers since.

The chief change is an interference with ossification leads to weakness of the bones, fractures and infractio areas where growth is taking place. The subsequent tr imposed on the original pathological process gives a co cated picture which is difficult to interpret. In additio changes vary in different bones and in different parts same bone, largely as a result of the rate of bone growt the degree to which these bones are used. For these reaso manifestations of scurvy are more severe in growing an than in adults.

The first change is a cessation of bone apposition. periosteum gradually thickens, the cells composing it lo like fibroblasts. Resorption with accompanying osteo continues unchanged. As a result, the shaft, especially part at the metaphysis where remodelling normally place, becomes very thin and may disappear. Fracture occurs with considerable haemorrhage and damage t cartilage trabeculae and cell columns, producing an ar debris, haemorrhage and fibrin deposition—the *trümme* When this happens, the cartilage cell sequences are n much disrupted as is capillary penetration, which is now irregular. As the disease progresses, a mass of fibroblas cells develops in the metaphysis on the diaphyseal side o *trümmerfeld*, occupying the area usually filled with the s giosa. This zone is known as the *gerüstmark*. This ma cells is probably made up of inactive osteoblasts which accumulated where they would normally be calcifying spongiosa [46]. This concept is supported by the findin rapid new calcification in the *gerüstmark* after ascorbic administration [Menkin *et al.*, 47]. Stunted trabeculae

roduced, but this may be due to the local trauma as the bone ecomes weakened. Follis [48] found that if the limb was immobilized a cartilage lattice formed but was not calcified.

The fundamental cause of these changes is still a matter of dispute, but it would probably be generally agreed that they are due to the inability of the osteoblast to secrete a calcifiable matrix. The collagen content of bones and teeth is reduced during scurvy [Robertson, 49], but the blood Ca and P levels are unchanged [Todhunter and Brewer, 50].

*Vitamin E.* No change occurs in endochondral ossification n deficiency of this vitamin [Irving and Budtz-Olsen, 51].

## Endocrine factors

*Pituitary.* The effects of this organ have been widely studied, especially by the Becks school, using the recently available growth hormone. Hypophysectomy stops all endochondral bone sequences, the changes being like those in ageing normal animals [Becks *et al.*, 52; Walker *et al.*, 53]. On giving the growth hormone to normal animals, the growth sequences persist and the bones are larger and longer [Evans *et al.*, 54]. When the growth hormone is given to hypophysectomized animals, endochondral ossification is at once stimulated Kibrick *et al.*, 55] and an almost normal histological picture s produced which is however continued for the whole experimental period [Becks *et al.*, 56] so that skeletal maturity, in he sense of closure of epiphyses, is not attained [53]. It may e that the growth hormone acts directly on the bone, since Blumenthal *et al.* [57] found, after administering it chorioallantoically to chicks during the last half of embryogenesis, hat there was a great increase in the weight of bones and in nitotic activity. As is well known, overactivity of the gland roduces clinically excessive bone growth in the conditions cromegaly and gigantism.

The interplay of hormones has also been studied. Thus testosterone mitigates to some extent the effects of hypophyectomy [Simpson *et al.*, 58]. When thyroxine is given to the ypophysectomized animal, the cartilage plate, sealed off by

bone as a result of lack of growth hormone, is now completely removed and the epiphysis closed [Asling *et al.*, 59].

*Thyroid.* Thyroidectomy causes a slowing in the rate of bone growth and differentiation. Endochondral ossification goes on, but at a very slow rate, so that the bone of a 72-day-old rat has the appearance of that of one 15–20 days old. Growth hormone stimulates growth but not differentiation of the tissues of the thyroidectomized animal, whereas if thyroxine is given, both growth and differentiation occur [Becks *et al.*, 60]. Long-continued overactivity of the gland produces osteoporosis, and the administration of the extract to normal animals causes an increased Ca excretion [Albright *et al.*, 61].

*Adrenals.* Adreno-corticotrophic hormone has been shown to retard chondrogenesis and osteogenesis in the normal rat [Becks *et al.*, 62] and also in the hypophysectomized rat given growth hormone [Becks *et al.*, 63]. It has hardly any further effect upon the bone changes in hypophysectomized animals. In Cushing's syndrome osteoporosis is common, and it is of interest that Albright [64] in his Harvey lecture predicted the existence of an anti-anabolic factor in the adrenal cortex some years before cortisone was isolated. When rats were both adrenalectomized and hypophysectomized, the bone picture was similar to that after hypophysectomy and the response to growth hormone was the same as if adrenalectomy had not been done—no increase in sensitivity was found [Simpson *et al.*, 65]. Follis [66] reported that cortisone, when given to growing rats, caused a dense zone of spicules of calcified cartilage matrix at the growing end of the bone which he thought was caused by a disturbance in the normal osteolytic activity, but in mice, rabbits and guinea-pigs no such action occurred. The reaction of the rat is thus in some respects anomalous.

*Oestrogens.* The action of oestrogens on bone is of great interest. It was first noted by Kyes and Potter in 1934 [67] that the female pigeon during the egg-laying period developed an excess of spongy bone in the marrow cavity of the long bones (medullary bone). When the Ca was needed for egg-

ll formation the excess bone was rapidly removed. During s time, from pre-ovulation till the eggs were laid, the blood rose to high figures. This effect could be reproduced in le pigeons and also in mice (female and male) by the ministration of oestrogen [Gardner and Pfeiffer, 68]. Male rmones had no such effect and the removal of the pituitary parathyroids did not prevent the hypercalcaemia [Riddle d McDonald, 69]. The local application of oestradiol to ps of bone implanted in the brain of litter mate mice was t followed by any bone change [Barnicot, 32].

The blood Ca, P and phosphatase of mice do not change in el when oestrogens are given. Urist *et al.* [70] investigated action of oestrogens in other animals, and of those they ted found young rats the only ones to be affected, but in a her different way. Here the resorption of cartilage and new ne was prevented so that a very large spongiosa developed. a later paper [Budy *et al.*, 71] it was found that the effect uld be obtained in both males and females, that no effect s obtained in rachitic animals and that desoxycorticosterone d probably testosterone did not produce any bony changes. n humans it is stated that the long-continued administra- n of oestrogen is followed by hyperossification [Cooke, 72], d Albright *et al.* [73] have reported that defective bone wth and osteoporosis could be found in both sexes in mary gonadal insufficiency. It would appear, however, that principal effect of oestrogens in bone formation is con- ned with egg-laying in birds, and the reason for a some- at similar action in some mammals is not very evident, as appears to fulfil no physiological need. In pregnant mice ther the foetal nor maternal skeletons show any medullary ne formation.

*Parathyroid glands.* These organs have the most profound ntrolling influence upon Ca and P metabolism. The general cts are discussed in the section on the blood Ca, and here y the histological changes in bone will be considered.

As far as the present writer knows, no description has en given of the effects of ablation of the glands on bone,

presumably because the experimental animals live so short a time after the operation.

In hyperparathyroidism changes are seen in bone which are in essence a destruction, an osteoclastic reaction predominating. Osteocytes and osteoblasts are destroyed in some animals. Snapper [74] summarized the bone changes in clinical cases as: proliferation of osteoclasts which erode bone with resulting decalcification, and proliferation of fibrous tissue in the Haversian canals; cyst formation, and formation of giant-cell tumours—osteoclastomas. Recently Heller *et al.* [6] have analysed the cell changes in rats after single toxic doses of parathyroid extract, and showed how, ranging round a central spindle cell, modulations (with little mitotic activity) could take place between all the cells concerned with bone formation and destruction. It is still undecided if parathormone acts directly on bone or if this dissolution is the result of changes elsewhere. Barnicot [75], using his implantation technique with parathyroid gland and bone, found that resorption and an osteoclastic response occurred in bone in contact with the gland; when transplants of pituitary, thyroid or adrenal gland were used instead, no change occurred.

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CHAPTER XI

# Bone – Chemistry and Physics

No attempt is made in this chapter to deal exhaustively with the chemistry and physics of bone. This would involve a great deal more space than available and has in any case already been done by a number of writers [Neuman and Neuman, 1; McLean and Urist, 2; Dallemagne, 3; Carlström, 4]. Only salient points which seem pertinent to the physiology of Ca metabolism will be discussed and the writer has in general followed these authors in his presentation.

Bone consists of an organic matrix, collagen, which is fibrillar in nature, and an amorphous ground substance which contains a mucopolysaccharide, probably chondroitin sulphate; in this organic framework are branching bone cells or osteocytes. About one-third of the bone mass is composed of minute crystals embedded in the organic matrix; these crystals contain chiefly Ca, phosphate and carbonate and confer on bone its hardness.

The organic matrix of bone is similar in general to that of other connective tissue and it may be asked why calcification is restricted to bone. This restriction is at times overruled, but under normal conditions the presence of bone-forming cells or osteoblasts is peculiar to bone only. In addition, the 'local factor' is postulated as conferring calcifiability on bone tissues.

The crystals of bone are microscopic in size and can barely be seen with the electron microscope. Authors agree that they are 25–30 Å thick, approximately 400 Å long and nearly as wide [Robinson and Watson, 5]. They are found in close association with the collagen, lying on the characteristic doublet banding of the fibres with the long crystal axis

arallel to the longitudinal direction of the fibre. The surface rea of the crystals is enormous, about 100 m.² per g., which ould mean a total surface area of over 100 acres for the one crystals of a 70-kg. man. This gives strong support to he conclusion that the available bone and plasma are in ynamic equilibrium.

The composition of these crystals has proved very hard to etermine, although X-ray analysis undoubtedly shows that he basic lattice structure of the bone salt is an apatite, but his is not to be regarded as a compound but more as an ar-ngement in space of the ions found in the mineral. The crystal f the bone salt is roughly hexagonal, with the *c* axis of the nit cells oriented in its long dimension. This unit cell is a nceptual configuration and has no independent existence. t is the simplest arrangement of the atoms, treated as points, at embraces all the different ions found in the crystal, in e same ratios (with the smallest common multiple) and in e same spatial relationships in which they are present in the ystal' [McLean and Urist]. When these units are outlined imaginary lines and extended through the crystal structure, ey give the three-dimensional crystal lattice. The unit in a oss-section of the crystal is a two-dimensional parallelogram th four equal sides of 9·4 Å in length, the *a* axis of the ll. In three dimensions, the unit cell is a six-sided right ism and the other dimension or *c* axis measures 6·9 Å. he *c* axis is oriented in the long dimension of the bone salt ystal.

The crystallites of bone salt are roughly hexagonal in shape th columns of Ca and oxygen arranged at the intersections the hexagons and of $OH^-$ ions running in columns down e middle of the hexagon, the hexagons also being packed th phosphate. 47·3 per cent. of the unit cells are on the sur-ce of the crystal (of dimensions as stated above), and about per cent. of the units have two faces exposed.

The nature of the bone salt has been much investigated and still a matter of dispute. One great trouble is that the par-les being so small, adsorption can easily occur and this

alters their chemical composition. Furthermore, freeing the bone of its organic components changes the nature of the solid phase. Even water will do this. A favourite method is to use KOH in boiling anhydrous glycerol, but here the water in bone will give an alkaline solution which will change tricalcium phosphate hydrate (which Dallemagne and his colleagues think is the solid phase of bone) into hydroxyapatite. Calcination, also often used, causes the crystals to grow and may induce reactions between apatite and ions outside the lattice. Thus it will be seen that the problem is no easy one.

Armstrong [6] has given a good summary of the possible concepts. These are that bone salt is: 1, carbonate hydroxyapatite, 2, tri-calcium phosphate hydrate, or 3, hydroxyapatite.

1. Carlström has put forward evidence that the concept of carbonate apatite with carbonate present in the crystal lattice is not correct and that the carbonate is present as a surface component or a separate phase, the former more probable. Neuman and Neuman have summarized the literature which is against this hypothesis. They prefer to talk of carbon dioxide and consider that this is located at the crystal surface and not present as a separate phase.

2. The tricalcium phosphate hydrate hypothesis is largely that of Dallemagne and his school and they have brought forward a great deal of evidence in favour of it [7]. It implies that the carbonate is present as a separate phase as $CaCO_3$, which is still unproved. The chief reason why they favour tricalcium phosphate hydrate is that hydroxyapatite has a molar Ca : P ratio of 1·66, whereas that of bone is usually lower, about 1·5, but varies with the dietary intake of the minerals. The molar Ca : P ratio of tertiary phosphate is 1·50. However, it seems doubtful if the methods used by Dallemagne are applicable with the accuracy he assumed. Thus all poorly crystalline apatite-like precipitates, irrespective of their composition, give indistinguishable X-ray diffraction patterns, and refractive indices of precipitates of crystallites of colloidal dimensions are of no value. Neuman has proposed that hydronium ions exchange for Ca ions in the surface layer of the

patite unit cell and that this and also $Na^+$ exchange for $Ca^{++}$ n account for changes in the Ca : P ratio.

3. It is generally agreed that the basic lattice structure of one is an apatite and hydroxyapatite is taken as the prototype. It is quite clear that bone salt is not simply this, but uch work has now been done showing that surface phenoena, due to the very large surface presented, can explain any of the figures which might be considered discrepant. The bone crystal can be pictured as consisting of 'a surface dration shell containing non-specific boundary anions in pid equilibrium with the surrounding medium; an inner, ystal surface containing more or less specific cations and ions also in equilibrium with the solution (or the hydration er); and interior ions with a slow but measurable equiliation with the outer layers (recrystallization)' [Neuman and euman.] Carlström pictures it as 'built up from a core of droxyapatite covered by a shell of different ions and water'. An ion can be in one of four positions in relation to hydroxyatite. It may be: 1. in an interior cell of the crystal lattice d thus in a relatively stable position, 2. in a surface position the crystal lattice, 3. adsorbed on the surface of the crystal 4. in solution in the hydration shell.

Surface adsorption can account for a good deal of variation the Ca : P ratio of the mineral, but there are limits beyond ich it is not possible to go. Thus a Ca : P ratio of 1·33 is not ssible as the crystals are not small enough to adsorb so ch P, one ion for every four in the crystal, and likewise gh Ca : P ratios cannot occur.

Surface exchange accounts for a great deal of the rapid upe of ions. Ionic exchange is now used by many chemists ng synthetic resins, clays, &c. In the case of bone, two es of exchange occur, isoionic and heteroionic. Isoionic change has been demonstrated with radio-isotopes *in vitro* ng dentin, enamel and bone, especially by Hodge and his ool. Crystal size, temperature, pH and many other factors ermine the final distribution of the isotope.

n heteroionic exchange an ion on the crystal lattice is

displaced by a different ion from the solution. Thus uranyl, strontium, sodium and hydronium ions will displace Ca and carbonate ions will displace surface phosphate. This displacement is to some extent specific. $Na^+$ and $H_3O^+$ whose ionic radii are close to that of $Ca^{++}$ can displace it mole for mole, but $K^+$ cannot as its ionic radius is greater. $Sr^{++}$ has an ionic radius almost the same as $Ca^{++}$ and exchanges with it on an equivalent basis. A mole of uranyl ion displaces two of $Ca^{++}$. The exchange of $F^-$ is unusual as it appears to be irreversible and more is taken up than can be explained by surface considerations. The citrate ion is peculiar in that 70 per cent of the citric acid in the body is found in bone, and it may account for as much as 1 per cent. of the fresh weight of bone. The size of the molecule excludes it from the apatite lattice. Exactly where the citrate is located is not very clear, but a considerable amount must be held on the surface of the apatite crystal. However, only portions of citrate are readily dissolved from powdered bone so that some must be held on entrapped surfaces.

The hydration layer is a true water of hydration and may be of considerable magnitude, as much as 80 per cent. in the case of synthetic hydroxyapatite. In fresh untreated bone the hydration is not so high, suggesting that some of the compact bone may be isolated from the circulating fluids.

Recrystallization of apatite crystals and of bone crystals occurs in an aqueous medium. Labelled Ca and P slowly equilibrate with the bulk of the mineral phase, far more slowly than in the process of exchange, and there is no release of these isotopes in short-term exposures to non-labelled buffer. Newly deposited bone salt recrystallizes much faster than old bone. As will be seen, both this process and ionic exchange play important roles in bone physiology *in vivo*.

**Calcification**

Attempts to explain this by solubility product relationships have not been successful. On ordinary physico-chemical considerations, for precipitation to occur, some solubility product

st be exceeded. This was the basis of the original phospha- theory of Robison. However, as Hodge has pointed out no solubility product has ever been demonstrated. Know- what we do now of the complexity of the composition of e crystals, this is not surprising. Levinskas [9] working n synthetic hydroxyapatite found the determination of a ıbility product impossible.

relationship does, however, seem to exist between calci- tion and the solubility product of $CaHPO_4$. Howland and mer [10] many years ago found that the presence or ab- ce of rickets in children had a relationship to the $Ca \times P$ duct of the plasma, the figures being for total Ca and P, e at that time no reliable method for determining Ca and P s existed. More recently the findings have been more re- d to the ion product $[Ca^{++}] \times [HPO_4^{--}]$ than to any other nbination. McLean and Urist [2] have drawn a graph of solubility product constants of $CaHPO_4$ and $Ca_3(PO_4)_2$ as ted to pH and the P content of the solution, and have super- osed the minimum ion concentrations at which calcifica- of hypertrophic rachitic cartilage occurs *in vitro*. At pH's ve 7·3, calcification is correlated with the solubility product $CaHPO_4$ and not with that of $Ca_3(PO_4)_2$ and thus the ion pro- t $[Ca^{++}] \times [HPO_4^{--}]$ appears to be the determining factor he process. This product defines the upper solubility limit Ca and phosphate ions in solution, but calcification can does occur (as in adults) at lower ion concentrations. The concentration determines the speed of calcification but its occurrence, the speed being determined by the colli- frequency. Thus it can be seen how Howland and mer's empirical formula, $Ca \times P$, is as reliable as the ion duct itself, since the ratios of $Ca^{++}$ and $HPO_4^{--}$ to the l Ca and total P are relatively constant.

euman and Neuman [1] stress our ignorance of the calci- ion process, but point out that from what little we do w, the process is not one of precipitation. The ion product a and phosphate ions in the serum at levels of 10 and g. per 100 ml. respectively, is far less than the solubility

product constant for $CaHPO_4$, yet calcification occurs fro
such serum without difficulty. Apatite crystals can grow at C
and phosphate levels well below the solubility product co
stant of $CaHPO_4$, and further it is hard to see how precipita
tion can result in the formation of a highly organized an
oriented mineral pattern. A process of 'seeding' must be co
sidered instead in which possibly the organic matrix of bo
can bind Ca or phosphate ions in the proper space relatio
ships for the apatite lattice, and on to these would be added th
other ions making up the complete crystal. As we shall se
there is evidence that such a process does initiate calcificatio

The exchange of ions *in vitro* and *in vivo* has been invest
gated by a number of workers. Much of this has been do
using $P^{32}$, but the handling of this element in bone and th
conclusions drawn apply in most cases equally well to C
Thus Falkenheim *et al.* [11] found that one-fifth of the P
bone could be exchanged *in vitro*, using $P^{32}$, and considere
that this must represent P on crystal surfaces. In a later pap
[12] the same was reported for Ca using $Ca^{45}$. Dawson [1
stated that 33 per cent. of the Ca of unashed bone wa
exchangeable.

The exchange of Ca and P *in vivo* has also been investigate
with radio isotopes. Neuman and Riley [14] using $P^{32}$ realize
that the rapid uptake *in vivo* was not due to normal growt
but to 'exchange-adsorption' between the P of the plasma an
that on the surface of the bone crystal, and was no index
growth changes or P deposition. However, if $P^{32}$ was given
*vivo* and the animal kept for longer times before sacrifice, th
$P^{32}$ became less desorbable, indicating that recrystallizatio
had occurred as well [Neuman and Mulryan, 15]. The sam
was found by Singer and Armstrong [16] and Tomlin *et a*
[17], using $Ca^{45}$. The latter workers reported that the inte
stitial replacement of Ca in previously deposited mineral wa
very slow and that there was a non-exchangeable accretio
involving about 1 per cent. of the total cortical bone per wee
Arnold and Jee [18] found that $Ca^{45}$ was rapidly removed t
non-exchangeable positions by crystallization and recrysta

tion, where it was not available for removal by ion-hange techniques, half of the Ca being so dealt with after hour.

Thus one has to picture the two processes of exchange and rystallization occurring side by side to account for bone wth and maintenance. In adult animals both processes bably account for the appearance of isotopes in bone, but young animals the formation of new crystals is quantita-ly very important. The site of deposition of isotope is erned by hydration, density and age of crystals, and fluid hange. The greatest concentrations of radioactivity are nd in young bone and in the most recently deposited eral, such as round Haversian canals [Arnold, 19]. The e is seen in sections *in vitro* and is thus largely due to the sive physico-chemical processes of exchange and recrystal-tion. Rachitic bone, in which skeletal maturation does not ur, likewise exhibits the high reactivity seen in growing e. In older bone, the uniform diffuse distribution seen in io-autographs of young animals does not occur. In estab-ed bone the circulation is poor and limited sometimes to small canaliculi connecting osteocytes. In addition, the ration of older bone is less, though it is not known through t agencies the hydration shell becomes reduced. Thus a ller and smaller fraction of bone, as the skeleton matures, n equilibrium with the body fluids. While nearly 100 per . of the skeleton of the young rat is available, this falls to er cent. as the animals become adult. Kornberg [quoted by man and Neuman, 1], using $Na^{24}$, found the available tion of the adult human skeleton to be 30 per cent. How-, it must be realized that in one small area of bone the of recrystallization and exchange may vary tremendously, to the various factors mentioned above.

hanges in the composition of bone can be quite satisfac-ly accounted for by the large crystal area which can carry excess of ions. Carlström [20] considered that the bone tallite surface could carry an excess of 1 to 3 Ca ions and 2 phosphate groups, and this could account for all Ca : P

ratios found by analysis; chemical analysis of bone was o very restricted value in the interpretation of crystal structure It is now well known that the inorganic composition of bon can be greatly altered by changes in the diet or blood chemis try, the most effective way being to alter the animal's acid base balance. The bone represents a great reserve of alka which supplements the buffers of the blood in maintaining normal pH. In acidotic states the bone has reduced $CO_2$ con tent, often the residual Ca : P ratio (after allowing for CO loss) is unchanged but the total Ca : P ratio may be altered

Shear and Kramer [21] found many years ago that newl calcified bone was low in $CO_2$, and in such areas the Ca : ratio was also low. In rats at the age of about twenty day the Ca : P ratio of the bone rose and at the same time the CO began to increase to the adult level [Neuman and Neuman Sobel and his colleagues in a series of important investiga tions have studied this matter further; their work has bee recently summarized [22]. They estimated particularly th $PO_4 : CO_3$ ratio in bone, and found [23] that high Ca : P rati diets caused higher $CO_3 : PO_4$ bone ratios than in animals o low Ca : P ratio diets. The $PO_4 : CO_3$ ratio in bone was relate to the $P : CO_2$ ratio in serum and the conclusion was draw that the composition of the diet was reflected in that of th blood serum and so in that of the bone [24]. The mechanis of this process is probably at least in part an exchange carbonate for surface phosphate, the process described heterionic exchange by Neuman and Neuman [Hodge, 25 Posner and Stephenson [26] in confirmation of the work Sobel *et al.* consider that 6 per cent. of the phosphate ion an inclusion in the entrapped surfaces of the hydroxyapati crystal and that such inclusions depend on the individual co centrations of phosphate and $CO_2$ in the serum.

Dallemagne [27] has postulated further that the very lo Ca : P ratios in young bone may be due to phosphate io attaching themselves to preosseous organic matrix.

It was mentioned above that bone has a buffer-like action maintaining the composition of the blood. This is true on

hin certain limits, and as far as Ca is concerned acts in ıcert with certain biological reactions such as osteoblast d osteoclast activity and renal action. However, it has been own that the injection or removal of large amounts of Ca m the circulation causes but little change in blood Ca con- ıtration [28]. As pointed out by Neuman and Neuman, this due to the constant dynamic equilibrium between the bone stals and the blood, and to the fact that the surface mineral exceeds the mineral content of body fluid. The blood of a -kg. man contains 0·25 g. Ca, while the bone surface Ca, culated as 10 per cent. of the available Ca, calculated in its n as 20 per cent. of the available skeleton, is 42 g. Thus the ysico-chemical reserve of surface Ca is nearly 200 times the ıount in the circulating blood.

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## CHAPTER XII

# Enzymes and Bone Formation

this chapter the emphasis may appear to be more on P than a, but since the two are intimately related it is clear that P position also involves Ca. The intervention of enzymes in one formation was first postulated by Robison in 1923 [1] hen he found phosphatase in bone. There are at least two hosphatases in the body, designated from their optimum H's, acid and alkaline phosphatase. The former is found in e prostate gland and has little to do with calcification. lkaline phosphatase, found in bone and other tissues, acts monoesters of phosphoric acid at an optimal pH of 9·4, erating inorganic P. Robison considered that this enzyme ted on a suitable substrate, the solubility product of calcium hosphate was exceeded and this salt was deposited in the ocess of calcification.

This theory as originally stated was soon found to be unisfactory. As already mentioned, calcification of bone does ot occur by the exceeding of a solubility product and preipitation from supersaturated solutions. Apart from that, itable substrates for the enzyme are none too plentiful and ose in blood are in the corpuscles. The optimum pH is very uch on the alkaline side. Besides this, phosphatase is found many other tissues which do not normally calcify.

It is worth while at this point to consider phosphatase in little more detail. There seems no doubt that phosphatase more associated with matrix formation than with calcificaon. Important accounts of phosphatase distribution in bone ve been given by Lorch and Siffert. Lorch [2] found no hosphatase in bone matrix or small-celled cartilage, but

present in hypertrophic cartilage (in rats not kittens), in osteocytes, Haversian canals, endosteum, inner lining of periosteum, and the linings of vascular and marrow spaces. Osteoblasts contained it, but marrow cells did likewise, so that she did not think that only the osteoblasts secreted phosphatase. Extra-cellular phosphatase was always found where ground substance was being deposited, but was absent in areas of resorption. Newly deposited bone salts were never found in areas where there was not some extra-cellular phosphatase, though the converse was not necessarily true.

Siffert [3] found that phosphatase activity and free phosphate localization did not invariably coincide and that phosphatase was more intimately related to preosseous cellular metabolism and to the elaboration of a matrix that was chemically calcifiable. The elaboration of bone matrix was always associated with phosphatase activity but could and did occur in the absence of calcification as in rickets. Calcification might occur later, in the absence of the enzyme. 'Left over' phosphatase, in cartilage remnants in the metaphysis, was still active but had no physiological significance. Bevelander and Johnson [4], in a study of the developing mandible, reported that phosphatase disappeared from the bone matrix as mineralization occurred. Morse and Greep [5] found in the normal epiphyseal cartilage that the content of phosphatase increased as the cells became more mature, the hypertrophic cell layer containing most, but the layer of primary calcification virtually none. In rickets the same was found in the cartilage cells, and osteoid also contained the enzyme.

Wislocki and Sognnaes [6] investigated the developing tooth and found the enzyme in sites of proliferation such as the developing dental pulp, tissue of the dental sac, periodontal membrane and gingival tissues. They also found phosphatase in small amounts in the predentin and dentinal fibres. In studies of amelogenesis Bevelander and Johnson [7] found phosphatase to develop in the ameloblasts just before matrix formation occurred and to remain in them during matrix formation and subsequent calcification. They also found [8]

at ribonucleic acid and phosphatase coexisted in terms of
me and localization in the odontoblasts and ameloblasts and
ggested that ribonucleic acid might be implicated in some
ase of the calcifying mechanism.

The behaviour of phosphatase in a number of conditions is
structive. Danielli *et al.* [9] studied wound healing in normal
d scorbutic guinea-pigs. In normal animals there were two
ases of phosphatase activity in the healing tissue, one due
leucocyte invasion, the second probably to the differentia-
on of collagenous fibrous tissue. But if the animal had scurvy
ere was no second peak and collagen formation was absent
greatly reduced. Gould and Schwachman [10] found in
orbutic guinea-pigs that the phosphatase content of serum
d bone rose and fell in parallel, that of the other tissues not
ing significantly altered. It will be recalled that the osteo-
asts become inactive during scurvy forming the *gerüstmark*.
uring healing from scurvy the bone and blood phosphatases
se together, and Gould and Schwachman considered the
ood phosphatase to be derived from osteoblasts. Norris and
ohn [11] found after injection of Ra that the alkaline phos-
atase of both bone and serum fell, but that of soft tissues
as not affected. *In vitro* no effect on phosphatase was seen
that the phosphatase-forming cells were affected by the Ra.
uring rickets the blood phosphatase rises considerably as was
own many years ago by Kay. In this condition there is much
atrix formation but no calcification. Incidentally it is difficult
correlate these findings with the theory of Zetterström that
tamin D is a coenzyme for phosphatase, since during rickets
nen calcification is in abeyance due to vitamin D lack, phos-
atase is most active especially in blood, while in scurvy,
nen there is no lack of vitamin D, phosphatase may disappear
om bone completely.

It may thus be concluded that phosphatase is concerned,
rtainly in bone and probably elsewhere in the body, with
atrix formation and its association with calcification in bone
probably incidental.

Some other quite different roles have been assigned to

phosphatase. Thus Neuman *et al.* [12] found that calcificatio
*in vitro* was inhibited by phosphoric esters if phosphatase wa
inactivated; they considered that phosphatase removed este
P which acted normally as an inhibitor of calcification. Di
Stephano and Neuman [13] reported that adenosine triphos
phate was an inhibiting agent to calcification *in vitro* since C
formed a relatively undissociated complex with it; they attri
buted this inhibiting action to the fact that phosphatase acte
very slowly on ATP. While these may seem rather far-fetche
theories, it is true that after the enzymes of bone have bee
destroyed, *in vitro* calcification will not take place in medi
containing organic sources of P, but only if the P is inorgani
[Waldman, 14].

Robison was led to postulate a second mechanism on to
of the phosphatase one when he found that certain procedure
stopped calcification of hypertrophic cartilage *in vitro* but ha
no effect upon phosphatase activity [Robison *et al.*, 15]. Th
researches into this second mechanism have taken two courses
one into the enzyme aspect and the other a search for a specifi
calcifiable substance in epiphyseal cartilage (the local factor)

It must be explained at this point how calcification *in vitr*
has been studied. The rachitic epiphyseal cartilage of the ra
has been used; when removed and incubated in appropriat
media, rapid calcification occurs in a manner that appear
identical with natural healing *in vivo*. Thus it is not ossificatio
that is being observed but calcification of cartilage. So far i
has not proved possible to cause rachitic osteoid to calcif
*in vitro* [Bloom, 16], and Bailie and Irving [17] give reasons fo
supposing that such calcification could not occur save in tissu
culture.

**Enzyme systems in calcification**

The role of glycogen in calcification appears to be signifi
cant. Only the hypertrophic cartilage cells contain it and the
correspond closely with those that calcify. If the glycogen i
removed with saliva, calcification will not occur, but can b
restarted with glucose-1-phosphate [Marks *et al.*, Marks an

norr, 18]. Marks *et al.* think that glycogen has to do with the eparatory stages of inorganic salt deposition in cartilage and e influence of glucose-1-phosphate is consistent with its sition in the glycolytic cycle. Cobb [19] confirmed the findgs of Marks *et al.* with regard to glycogen, in that the cells owing the most rapid formation of calcifiable matrix conined phosphorylase and had contained glycogen; the glycogen ight be concerned with the production of bone matrix or teoid or the alteration of cartilage matrix prior to calcificaon. Follis [20] also found that the calcification of rachitic rtilage occurred in the matrix of cells containing glycogen. lbaum *et al.* [21] have found adenosine triphosphate (ATP) epiphyseal cartilage and that Ca prevented its disappearce from incubated cartilage slices. These authors think it ssible that ATP is the substrate for phosphatase postulated Robison and that ATP, rather than glycogen, is the limiting ctor in calcification, but this does not appear to be in line with e findings of DiStephano and Neuman [13].

Turning to the enzymes involved in calcification, here the sition is by no means clear, since calcification goes on quite ell in certain media when all enzymes have been inactivated, ough again in certain other media, enzymes are needed. utman and Yu [22] investigated the actions of various enmic inhibitors. They stated that in the presence of phloridn, calcification from inorganic salts was stopped. Phloridzin hibits phosphorylase and prevents the formation of glucosephosphate. The addition of glucose-1-phosphate or any other ters concerned with subsequent stages of the glycolytic cycle ould allow calcification to proceed in the presence of hloridzin. Iodoacetate inhibits 1-3-diphosphoglyceric aldeyde dehydrogenase and blocked the calcifying power of all ters higher in the cycle and of inorganic P, but not that of phosphoglycerate. Fluoride, which blocks enolase, inhibited e calcification with inorganic P, 3-phosphoglycerate and all ters higher up in the cycle. From this it might appear that lcification chemistry follows the same pathway as found other tissues. However, no substrate for phosphatase is

provided at the end of the cycle, the P reappears as inorganic P and, as McLean and Urist state, the system runs into a dead end. Although when P is supplied as $\alpha$ or $\beta$ glycerophosphate, phenyl phosphate or creatine phosphate, there is good calcification in the presence of all these inhibitors (and these esters are all acted on by phosphatase) they are not in the main pathway of glycogenolysis.

Furthermore, calcification can still occur when enzymes are destroyed. Waldman [14], using a variety of methods which inactivated all enzymes, found that calcification would still proceed from inorganic phosphate media, but no longer from organic ester sources. He therefore concluded that enzyme systems were not necessary for calcification. Hiatt *et al.* [23] also found that certain inhibitors inactivated calcification from organic but not inorganic sources (though heat prevented calcification from inorganic phosphate) and were puzzled by the role of inorganic P which, while appearing to traverse the glycolytic cycle, could still cause calcification when the cycle was inactivated. However, the inhibitors used would, in higher concentration, also stop calcification from inorganic sources and they concluded that under the condition of their experiments calcification was dependent on an enzyme system. Goldenberg and Sobel [24], arguing from the results of F and cyanide inhibition, also considered the mechanism of calcification from both inorganic and organic media to be the same and not two discrete processes. If the glycolytic enzyme system does play a part in calcification, which it may well do in the living organism, it may be concerned with moving phosphate across cell membranes, or against concentration gradients, and the high-energy phosphate bonds formed may play some important role in the process. This is at present pure speculation, and only further research will give us the answer.

Ca has not been mentioned as playing a part in the above processes. It is, however, involved with the 'local factor' which is supposed to be present in calcifiable cartilage.

*The local factor.* There is now strong evidence that the substance which takes up Ca is a mucopolysaccharide and prob

bly chondroitin sulphate. Thus Boyd and Neuman [25] con-
dered that the binding of Ca by cartilage was connected with
s sulphate content, implicating chondroitin sulphate. They
rther stated that phosphate was taken up only by cartilage
hich contained an appreciable amount of Ca. Bélanger [26],
n auto-radiographic evidence, suggested that there was a
lationship between the local distribution of Ca in cartilage
calcified after decalcification and that of labelled sulphate.

Sobel and his colleagues have done much work, especially
n the reversibility of calcification. They found that many ions
ch as Sr inactivated calcification *in vitro* [Sobel *et al.*, Sobel
nd Hanok, 27], but that this inactivation could be reversed
y shaking the cartilage in Ca-containing solutions. They con-
dered there was a competition for the constituent necessary
r calcification which in their opinion was chondroitin sul-
hate and that the first step in calcification was a combination
ith Ca. In a later paper [Sobel and Burger, 28] they used a
nthetic collagen chondroitin sulphate complex. This took
p Ca like rachitic cartilage to the extent of an ash content of
) per cent. dry weight and the deposited salt had an apatite
iffraction pattern. They considered that a Ca complex first
rmed which reacted with phosphate to form an apatite salt
nd the process was then repeated. This view was supported
y the finding that mineralization only occurred if the com-
lex was shaken first with Ca salts and then with phosphate,
ut not in the reverse order.

DiStephano *et al.* [29], using chromatographic analysis,
ound a new spot in material from rachitic rat cartilage which
ppeared to be a stable ester of hexosamine. Procedures which
topped calcification caused the disappearance of this sub-
tance which they considered acted as a template for crystal-
zation of the inorganic material during calcification.

Evidence of another kind also supports this concept. Histo-
gically, mucopolysaccharides show metachromasia and there
s much evidence that this staining reaction is strong where
alcification is going to occur. Sylvén [30] and Levine *et al.* [31]
onsidered the metachromatic and basiphilic staining material

in osteoid, bone and cartilage to be acid mucopolysaccharide, almost certainly chondroitin sulphate. Rubin and Howard [32] found the metachromasia to increase in areas of cartilage about to become calcified and in areas about to become bone. This suggests either the formation of a new mucopolysaccharide or a change in that already there, conferring calcifiability on the matrix. Howard [33] showed that the disappearance of metachromasia and of the periodic acid-Schiff reaction with calcification was due to masking with bone salts, and these reactions could be made to reappear if careful decalcification was carried out. Wislocki and Sognnaes [6] also found metachromasia and basiphilic staining in dentin and enamel, indicative of the presence of an acid mucopolysaccharide.

As far as the specificity of these staining reactions in bone is concerned, Heller-Steinberg [34] has sounded a note of caution, as minor variations in technique could change the sites of metachromasia or intensify them. All acid radicles in a tissue can be made to react metachromatically and decalcification increases metachromasia. She does not feel that under these circumstances metachromasia necessarily indicates the presence of chondroitin sulphate.

Thus in the case of bone, the possibility of a template action of chondroitin sulphate is not yet certain. The sulphate content of the organic matter of cartilage is at least six to eight times that of bone [Logan, 35], and Rogers [36] reported very small amounts of mucopolysaccharide in ox cortical bone. It is, however, of interest that Sobel *et al.* [37] have found after demineralization of bone with versene that the bone can be recalcified by shaking first in a $CaCl_2$ solution and then with phosphate.

Another type of evidence supports the concept of acid mucopolysaccharide acting as a template in cartilage. Miller *et al.* [38] found that preliminary exposure of the cartilage to metachromatic or other basic dyes inhibited subsequent calcification in a calcifying medium. But if both Ca and phosphate were present with the dye, no inhibition occurred; if Ca or P only were present, then the dye inhibited. They concluded

at the dye reacted with an acid mucopolysaccharide essential r calcification, probably chondroitin sulphate. Incidentally, hile the dye inhibited calcification from inorganic media, it d not stop calcification from organic sources. Sobel and urger [39] have also reported the inhibitory nature of these es.

Exactly how all these results fit in with the enzymic system at present not clear. The fact that the dye inhibition does ot affect calcification from organic media seems to suggest en more strongly that calcification from inorganic and ganic sources are by means of different processes, or that lcification caused by the glycolytic cycle does not involve ondroitin sulphate. In spite of all these queries, it must be lmitted that there has been a great advance since Robison's iginal work, and much of the calcification mechanism is now derstood.

A final word should be said about the citrate in bone. ickens in 1941 [40] showed that over two-thirds of the citrate the body was in bone. In the previous chapter mention was ade of its possible position in the apatite crystal. What exact le citrate plays in bone is not known. Dixon and Perkins [41] und that enzymes existed in bone capable of causing a high trate content. The amount of citrate in different parts of bone as in inverse ratio to the metabolic activity of these parts. arathyroidectomy caused a slow fall in bone citrogenase, but ad no effect upon the bone citrate content, and the lowering the blood Ca was not due to changes in the enzyme activity the bone [42]. Steenbock and Bellin [43] reported that ysiological doses of vitamin D increased blood and bone trate (and that in several other tissues); bone citrate was ot reduced in rickets, but seemed to have some relation with e Ca status of the animal. Freeman and Chang [44] found at vitamin D in large doses produced a parallel change in asma Ca and citric acid, suggesting it influences the meta- olism of both substances. It has also been reported that para- yroid hormone mobilizes citrate together with Ca. Thus trate appears to play some active part in bone metabolism.

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## CHAPTER XIII

# The Calcium Metabolism of the Teeth

HE teeth consist of three different calcified tissues: enamel, ntin and cementum. The bulk of the tooth consists of ntin, the crown is covered by enamel, and the roots by mentum.

Enamel is the hardest tissue in the body, containing 1·2 to 0 per cent. water, 0·2 to 0·8 per cent. organic matter and 95 97 per cent. inorganic matter [1]. One analysis gives the Ca ntent of the ash to be 39·98 per cent. and the P content ·42 per cent. [Murray, 2]. Sobel *et al.* [3] and Trautz *al.* [4] find that the calcified elements show an X-ray ffraction pattern of apatite. The protein of enamel is -keratin [Hess *et al.*, 5].

Dentin is a more elastic structure. It contains 10·8 to 15·7 r cent. water, 20·3 to 22·4 per cent. organic matter and 61 to per cent. inorganic matter [1]. Murray gives the Ca and P ntents of the ash as 39·83 and 19·04 per cent., respectively. he mineral matter has the apatite pattern. The protein of ntin is a collagen [Hess *et al.*, 6]. Burnett and Scherp [7] sted the 'accessibility' of the protein by coupling it with azonium compounds and found the protein unreactive till calcification was begun, when the amount of azo-protein rmed was proportional to the weight loss.

Cementum is chemically and physiologically like bone. Zipkin and Piez [8] found citrate in both enamel and dentin; e dentin contained around 0·9 per cent., about eight times as uch as in enamel; very insoluble in both tissues.

The formation of enamel and dentin during development of e tooth are quite different processes. The enamel is formed

by the enamel organ, a complicated structure in which the active cells are the ameloblasts. An organic matrix is laid down first, and is gradually thickened by increments to its final width. As the matrix becomes mature it is calcified, and while the Ca and P increase the moisture and organic parts decrease [Weinmann *et al.*, 9]. Deakins [10] found that Ca P and $CO_2$ contents increased linearly and in a constant ratio as the enamel matured, a five times increase in inorganic content occurring from softest to hardest enamel. Water and in organic material exchanged in equal proportions and the final calcification of enamel was not just a 'drying up'. As a result of the high degree of mineralization of enamel, the fully calcified tissue is lost in decalcified histological preparations unless special precautions are taken to preserve the fine organic matrix. The processes of matrix formation and calcification appear to be closely linked, even at an early stage, since interference with calcification stops matrix formation (Irving, 11] which is not true of bone or dentin. Some workers [e.g. Nuckolls *et al.*, 12] have divided the calcification process into primary and secondary stages, but this seems to the present writer unnecessarily complicated.

How the enamel is transformed into its final form is still not understood, but it appears certain that this must be carried out by the enamel organ only and not partly by the pulp as claimed by some. If the ameloblasts are damaged the matrix does not mature properly [Chase, 13; Weinmann, 14; Irving 11] and it remains acid insoluble in histological preparations. Thus the enamel organ has probably one of the most diverse roles of any collection of cells in the body: it forms matrix, calcifies it, removes water and organic material, and in the case of some rodents, finally lays down a pigment layer on the incisor tooth.

Dentin is formed by cells lining the pulp, the odontoblasts. They probably change the material of the Korff fibres, formed in the pulp, which pass into the dentin between them, into a collagenous matrix, which they then calcify. Calcification is normally delayed for a definite period, twenty-four hours in

e case of the rat incisor, the uncalcified matrix being called redentin, staining with eosin in decalcified sections, the fully lcified dentin taking up haematoxylin. This change in staining reactions which accompanies calcification has, as in the se of bone, been attributed to some chemical change in the atrix [Weinmann and Sicher, 15].

Three types of material have been studied: fully erupted eth, teeth before eruption, and continually erupting teeth of nimals such as rats. The methods have been chemical, physical, using X-ray (Grenz) analysis, and histological, both conentional and radio-autographic, with $Ca^{45}$, $P^{32}$, and other otopes. Ground sections are often employed to avoid enamel ss during decalcification, but do not show the finer detail in llular and matrix structure. Although this monograph is rimarily about Ca, it is impossible to avoid mentioning results btained with P, since the two elements are so similarly treated.

It should be appreciated that the fully formed and erupted ooth is outside almost all vital influences of the body (with the xception of a few processes such as the formation of secondry dentin), and thus metabolically should be very inactive, his applying especially to enamel. Experimental results support this supposition. Thus Hevesy *et al.* [16] calculated that he human tooth exchanged about 1 per cent. of the dietary every 250 days. Hevesy and Armstrong [17] likewise reported hat the enamel of the permanent and erupted teeth of cats ook up little injected radio-P, about one-tenth of that of entin. Volker and Sognnaes [18] gave radio-P to an adult at by stomach tube; they detected 4·4 per cent. of the dose the teeth of which 91 per cent. was in the dentin and 2·3 er cent. in the enamel. Barnum and Armstrong found the ame [19] and considered that pathways existed for $P^{32}$ to go om the saliva to the dentin via the enamel, and from the lood to the enamel via the dentin. The main source of $P^{32}$ the teeth was from the blood-supply to the pulp. They ound that the P of rat molars was not completely removed y exchange after 116 days. In confirmation of the importance f the blood-supply, Gilda *et al.* [20] reported pulpless teeth

to take up much less $P^{32}$. Sognnaes and Shaw [21] have studied the uptake of injected radio-P in enamel and dentin of the erupted teeth of monkeys and found that there was a gradient in concentration of $P^{32}$, in the case of enamel falling from the exterior to the interior parts and rising from the internal to the external (nearest the pulp) dentin. They attributed these uptakes to salivary $P^{32}$ in the case of enamel and to the blood radio-P in the case of dentin. They also studied unerupted enamel [22] and considered that the very large uptake by this tissue was due to a high rate of exchange plus an active accretion of P accompanying the final growth of submicroscopic crystals.

*In vitro* work has shown that, as with bone, powdered dentin and enamel will adsorb $P^{32}$. Enamel has a slower rate than dentin and both have less adsorbing power than bone, possibly due to differences in size of the mineral crystal [Hodge *et al.* 23]. Thus in the erupted tooth the uptake of $P^{32}$ must, as in the case of fully mature bone, be largely a process of exchange and possibly recrystallization.

Much work has been done using the continually erupting incisor tooth of the rat. This, as would be expected from a continually calcifying structure, takes up radio-Ca and P with great readiness. Manly *et al.* [24] considered that there was a labile part of the tooth in equilibrium with blood $P^{32}$ and a stable fraction building up radio-P incrementally. In the rat incisor, 73 per cent. of the $P^{32}$ was in the labile fraction after one day, and 3 per cent. at twenty days after dosage. Carlsson [25], using $Ca^{45}$, found that the content of the isotope in the incisor rose continually up to twenty-four days after administration. But this interstitial metabolism is, in the case of dentin, very low compared to that of bone, although the $Ca^{45}$ returned to the circulation with the resorption of bone gives a persistent activity for up to ten weeks [Tomlin *et al.*, 26]. Armstrong [27] found in mature rats that the incisal uptake of radio-Ca and P given together was about equal in enamel and dentin.

The microscopic details of the incorporation of $P^{32}$ into

namel and dentin have been studied by Bélanger using radio-utographs [28]. In the case of enamel, a wide band of radio-P as seen at the dentino-enamel junction at the formative end f the tooth (in the transitional enamel stage), but not where ne enamel was first deposited, showing that newly formed natrix has to reach a certain stage of maturation before it is eceptive to mineral deposition, this stage being reached in a nort time after formation. During the process of general nineralization, the enamel took up less and less $P^{32}$, and nally, after optimal saturation, no more. In dentin, a thin adioactive line appeared near the pulp and slowly approached ne dentino-enamel junction, and eventually disappeared, leav-ng behind a diffuse distribution of radioactivity. Mineraliza-on of dentin occurred in a series of waves, and the $P^{32}$ became ncorporated, by dissolution or reconstruction of existing rystals, into more stable crystals.

The relationship between mucopolysaccharides and calcifi-ation appears to be similar in teeth to that in bone. Pincus [29] ound chondroitin sulphate in dentin, and Rogers [30] stated nat there was 0·1 per cent. hexosamine and 3·5 per cent. of otal reducing substances in dentin. Hess and Lee [31] reported ·64 per cent. of chondroitin sulphate in dentin, analytically ne same as in cartilage. Thus there is little doubt of the exist-nce of this substance in dentin. Engel [32] found histologi-ally a glycoprotein in many parts of the enamel organ, and lso in ameloblasts and odontoblasts in the early stages of ooth development prior to the formation of enamel and entin. Wislocki and Sognnaes [33] found an acid mucopoly-accharide in enamel and dentin. Both Burstone [34] and evelander and Johnson [35] reported a relationship between netachromasia and calcification in dentin, the latter workers ating that metachromasia gradually disappeared as calcifica-on proceeded. Greulich and Le Blond [36], using $C^{14}$, etected what was probably a carbohydrate which appeared at ne predentin-dentin junction at the same time as mineral rystals. They thought the crystals were bound to the collagen natrix by means of this carbohydrate. Bélanger [37], working

with radio-S, found this isotope in the ameloblasts and new enamel during the growth period. This, he stated, was synthesized to an acid mucopolysaccharide which appeared in the matrix before keratin and disappeared progressively. Thus there is a good deal of evidence that, as with bone, acid mucopolysaccharides are involved in the calcification of enamel and dentin, and they may act as a template for the crystallization of the apatite structure. It is also of interest that Engel [32] found glycogen in many parts of the developing tooth and associated structures.

The studies of dental enzymes have centred chiefly round alkaline phosphatase which is found in many parts of the developing tooth. In view of what has been said above about the functions of this enzyme, it is doubtful, at any rate in the opinion of the present writer, whether it has any direct connexion with the calcification of enamel and dentin, though it has been found in ameloblasts and odontoblasts while enamel and dentin were being elaborated [Johnson and Bevelander 38]. It has also been demonstrated in dentinal fibres and enamel prisms and very abundant in the growing pulp [Wislocki and Sognnaes, 33]. Symons [39] reported the appearance of phosphatase in areas where enamel would appear, but the odontoblasts had less than the adjacent pulp cells. The finding of phosphatase in the pulp, where the Korff fibres are formed lends support to the view that the enzyme is more concerned with matrix formation.

**The influence of diet and endocrine factors upon the teeth**

Leaving aside the production of caries, which is undoubtedly dietary in origin, the fully erupted tooth is outside the influence of dietary factors, but the developing tooth and the continually erupting incisors of rodents are very sensitive to dietary changes. Sobel and Hanok [40], working with rat incisors, have reported that, as with bone, the $PO_4$ : CO ratios of enamel and dentin are related to those of the blood serum and to those of the diet.

Ca, P or vitamin D deficiency, or parathyroidectomy, al

ave similar effects on dentin formation in continuously upting incisors, and Erdheim in 1906 was the first to report ie changes after parathyroidectomy. Histologically, the pre-entin becomes wider, often with vascular inclusions, and the alcified dentin is irregular and arranged in small calco-pherites (interglobular dentin). Becks and Ryder [41] were mong the earliest to report this condition in low P rickets. he Ca : P ratio of the diet is important in this respect. Gaunt nd Irving [42] found that diets of high Ca : P ratio affected ie bones more than the teeth, but if the ratio was low, the verse occurred, and the dentin ash was reduced. When tamin D is given to rats with low P rickets, the newly formed redentin is properly calcified, but that existing before the tamin was given remains unchanged [Irving, 43]. This illus-ates another difference between bones and teeth, the latter aving no reparative power. They also have no resorptive echanism and in Ca or P deficiency the pre-existing dental ssue is unaffected.

Several factors causing sudden changes in dentin calcifica-on produce a similar histological picture which has been nalysed by Irving and Weinmann [44] and called the calcio-aumatic response. The last formed dentin becomes hyper-alcified, the existing predentin is not calcified at all, and the ext formed dentin is hypercalcified for a short time. This is ometimes seen in the deciduous teeth of children caused by ie adventure of birth, and called the neonatal line [Rushton, chour, 45]. It is also caused by injections of fluorine [Schour nd Smith, 46], large doses of calciferol or parathyroid hor-one [Schour and Ham, 47; Schour *et al.*, 48], strontium jections [Irving and Weinmann, 44] and nephrectomy rving *et al.*, 49]. This response appears to be a non-specific action to changes in the Ca and P environment of the tooth. pplebaum [50] has shown by Grenz-ray analysis that the eas appearing histologically hypo- and hypercalcified are in ct radiolucent and radiopaque. The reasons for these changes re not understood, but they may be due to alterations in the alcifiability of the matrix.

Hypoplasia of the enamel in humans is usually caused b systemic disturbances during enamel matrix formation, to little enamel being formed [Diamond and Weinmann, 51 which may or may not be hypocalcified. In amelogenesi imperfecta, a rare congenital condition, the enamel matri forms but calcifies incompletely.

Several vitamins besides vitamin D have characteristic pro tective effects upon the tooth, but these probably affect matri formation rather than calcification. In vitamin A deficienc dentin formation is upset, the odontoblasts do not regress a they. should and excessive dentin is formed on the labial side of the incisor tooth [Wolbach and Howe, Schour *et al.*, 52] Enamel formation is deranged and the incisor loses its pigmen [Irving and Richards, 53]. The vitamins of the B group do no appear to influence tooth formation. In scurvy a characteristi change occurs, the formation of dentin ceasing and the las part formed being sealed off from the pulp by a hypercalcifie zone [Fish and Harris, 54]. The odontoblasts lose their abilit to make a calcifiable matrix and change to a structure like th *gerüstmark* of bone [Irving and Boyle, 35]. Both vitamin A an C thus affect tooth calcification as well as that of bone, and i a similar way, but little is known of the biochemical change underlying these effects. Lysine or tryptophane deficienc causes a change in the dentin very similar to that seen in C or P lack [56].

Some of the endocrine glands besides the parathyroids affec tooth formation, causing changes in calcification, matrix for mation or the growth sequences. Hypophysectomy produce a great retardation in the eruption rate, and as a result th rat's incisor has a folded appearance at the formative en [Becks *et al.*, 57], but dentin apposition continues so that th pulp cavity is almost obliterated [Schour and van Dyke, 58 The calcification of the tooth is not changed. Thyroidectom retards the calcification of dentin [Ziskin and Applebaum, 59 which becomes virtually uncalcified matrix, as shown b Grenz-ray analysis [Ziskin and Applebaum, 60]. Adrenalec tomy and gonadectomy interfere with dentin calcificatio

61, 62], but oestrogen administration has no effect on rat r mice teeth, which is of interest when its effects on bone re recalled [63].

One can conclude that while the fully formed and erupted ooth is outside influences exerted through the body, the form- ng tooth is very sensitive to changes in Ca and P metabolism nd is under the control of several vitamins and most endo- rine glands. It has been found that the dentin of rachitic rats esponds exponentially to vitamin D dosage in the same way s does the rachitic epiphysis [Irving, 43], and also that the ental changes after vitamin D dosage could be detected after wenty-four hours, four days before any change could be seen n the bones. The teeth have been largely neglected by physio- ogists, which is to be regretted since they react so quickly and pecifically to many diverse influences.

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## CHAPTER XIV

# The Excretion of Calcium

Ca is lost from the body by three ways: from the gut, the kidneys and the skin. The latter is the least important but a by no means insignificant route. Mitchell and Hamilton [1] found sweat to contain 2–7 mg. per 100 ml., and that even under minimal sweating conditions the daily loss could be 149 mg. four to 14 mg. per hour could be lost at high temperatures (Johnston *et al.*, 2].

The mechanism of Ca excretion by the kidney has been investigated by a number of workers. The constant presence of Ca in the urine had been explained by Albright and Ellsworth [3] as due to the threshold for Ca being normally 8·5 mg. per 100 ml. serum, so that with a value of 10 mg. the threshold would be always exceeded. This explanation is doubted by Robertson [4], who found the usual level of Ca excretion in a patient with a serum Ca level of 5·5 mg. per 100 ml. Wolf and Ball [5] thought there was no renal threshold for Ca and that the plasma Ca level was not regulated by renal function. In a series of important papers, Chen [6] and Chen and Neuman [7] have reported findings on the handling of Ca by the renal tubule. The Ca percentage in the glomerular filtrate was almost the same as that in an ultrafiltrate of serum (the ionized Ca fraction), reabsorption was an active process and over 99 per cent. of that filtered was reabsorbed. The small but constant amount of Ca excreted was considered to be an unionized complex, since the Ca clearance was increased by the injection of phosphate or versene. Chang and Freeman [8] also found that the injection of citrate greatly increased urinary excretion. If this concept is true it suggests that the kidney

is very efficient at handling ionized Ca and that the Ca lo in a form that the tubules cannot absorb.

The process of Ca reabsorption is different from that w acts on Na or K [Chen and Neuman, 7]. Ca reabsorptio depressed by phloridzin, dinitrophenol or Na azide, non which affects Na or K reabsorption. On the other hand, dia (a carbonic anhydrase inhibitor) increases Na and K excre but has no effect on that of Ca. Phloridzin and dinitroph inhibit phosphorylation reactions and the generation of h energy phosphate bonds, and both they and Na azide inter with the secretory activity of the tubules. Thus some kin metabolic energy is needed for the reabsorption of Ca.

The percentage of total Ca excretion which appears in urine has been stated by some investigators to be under nor conditions fairly constant, about 24 per cent. [Bauer *et al.* Robertson, 4]. Nicolaysen *et al.* [10] in a long study of o 100 men and women found the Ca in the urine, as a percent of the intake, to be between 20 and 25 per cent. Knapp [ likewise reported an average figure of 25 per cent., but a gr variation in his group of subjects. While the urinary Ca cretion was constant within individuals, it varied considera from person to person [10], and even in the same individ periods of change could occur for no apparent reason. Alt ing the Ca intake might or might not affect the urinary outp Seeing that the handling of Ca by the kidney is under control of a number of endocrine factors, this variability is surprising. Furthermore, the pH of the urine is a ruling fac since urine cannot act as a vehicle for Ca excretion if it alkaline, though in man this does not normally occur.

After hypophysectomy the urinary Ca excretion falls humans [Luft, 12], but this may not be due to the loss o direct action of the pituitary since adrenocorticotropic h mone, cortisone and desoxycorticosterone cause a negative balance [Luft *et al.*, 13] and cortisone increases the urinary a faecal Ca [Soffer *et al.*, Moehlig *et al.*, 14]. The thyroid h a marked action on Ca excretion in the urine and in hyp thyroidism there is a considerable Ca loss [Aub *et al.*, 1

obertson [16] has proposed that the renal threshold for Ca lowered, and that the excessive Ca loss causes by a *vis à onte* action a decalcification of bone. The parathyroids have een postulated to have a direct action on the kidney. This has een discussed in Chapter IX.

Before leaving this aspect of the subject, a word should be id on the renal action which has been claimed for vitamin D. arrison and Harrison considered that the vitamin caused an crease in tubular reabsorption of P and a rise in serum Ca nd P, A.T.10 and parathyroid hormone causing a fall in P absorption [17]. There seems to be some confusion about the fect of the reabsorption of P on the blood Ca level; if these sults are true, both an increase and a decrease in this reab-rption accompany a rise in the blood Ca. Crawford *et al.* [18] nsider that the results with vitamin D have been due to a ppression of parathyroid action since, if parathyroidectomy carried out, vitamin D causes a decrease in tubular P reab-rption. This finding makes the renal action of vitamin D ore consistent with that of the parathyroid. Cruickshank *al.* [19] found, after giving a large dose of vitamin D, that considerable amount was retained by the kidney, and they ought this might be connected with a tubular action.

The faecal Ca in man consists largely of unabsorbed food a. It was shown in Chapter III that, in addition, secretion of a into the gut undoubtedly occurs. Geissberger, Bellin and aszlo, and Staub [20] have injected $Ca^{45}$ into humans and und the isotope to be rapidly eliminated in the faeces in nounts of from 2·5 to 15 per cent. of the dose. Geissberger ated that four days later there was more $Ca^{45}$ in the faeces an in the urine.

From the evidence it seems legitimate to conclude that the man intestine can excrete Ca but may not do so always. The ndings of Steggerda and Mitchell [21] are conclusive on this int. They gave citrate to men and reported that the faecal Ca· ight amount to over twice the Ca intake. It is true that in ese experiments the conditions were unusual in that the urine ight have been alkaline (the pH of the urines was not

determined), but none the less the fact was established that intestine could act as an excretory pathway. Whether it c this under normal conditions in any significant sense is poss doubtful. McCance and Widdowson [22] gave humans gluconate intravenously and found no increase in faecal Johnson [23] and Welch *et al.* [24], studying patients v ileostomies and colostomies, found no evidence of Ca cretion in the gut.

Animal experimentation suggests that they too can exc Ca in the faeces. Thus rats given $Ca^{45}$ by injection excre considerable proportion of the dose in the faeces [Greenb L'Heureux *et al.*, Wallace *et al.*, 25], the latter two gro considering that the small intestine played a major r Rabbits have likewise been shown to excrete Ca in this w Sjollema [26] many years ago thought that Ca played a p in faeces formation in these animals. Cowell [27] cau uranium nephritis in rabbits and as a result the faecal exceeded the urinary plus food Ca. In herbivorous anim who habitually secrete an alkaline urine, faecal Ca excret would be expected.

Other animals have been shown to excrete Ca by this rou e.g. cats [Stewart and Percival, 28] and dogs [Greenwald a Gross, 29].

From the evidence cited above it may be concluded that man the kidney is the main excretory pathway, the Ca be lost possibly owing to the kidney tubule being unable to absorb unionized Ca complexes. The intestine is able excrete Ca and forms a reserve mechanism for this purpos

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## CHAPTER XV

# Extra-skeletal Functions of Calcium

In the previous chapters the description of the metabolism of Ca has centred round its functions and participation in the formation and reactions of the hard tissues. Ninety-nine per cent. of the Ca of the body is in the skeleton and teeth, but the remaining 1 per cent. plays an important role in cellular and other bodily activities, which will be briefly reviewed.

Ca has a number of general actions which have been largely investigated in protozoa and simple metazoa. It has a protective action against the toxic actions of other ions, especially Na and K. The anaesthetic action of Mg can be reversed by small amounts of Ca. Even in small concentrations Ca will protect against the toxic action of distilled water, and solutions containing only Ca salts are not harmful to many lower organisms.

Ca governs cell permeability, usually by reducing it. This has been found with plant cells and human erythrocytes, in which the leaching of salts is retarded by Ca; the rate of intestinal absorption of sugar is lowered in parathyroidectomized rats. Ca also maintains the semipermeability for water of membranes such as those of sea-urchins and plant cells.

The absence of Ca from the environment is most injurious, largely owing to the unopposed effects of other ions. Muscle will not contract in the absence of Ca, this applying to smooth, heart and striated muscle. On the other hand, this tissue is sensitive to the concentration of Ca. Ringer showed many years ago that a solution with an excess of Ca perfused through the heart caused contraction to cease in systole, and as mentioned earlier, McLean and Hastings have used the frog heart

as a method of estimating the level of free Ca ions. Skelet muscle is impermeable to Ca, but if it is cut so that Ca io can enter, violent tetanic contractions occur.

Ca is apparently essential for clotting reactions, since takes part in the coagulation of milk and blood. It has marked effect on the integrity of intercellular cementing sub stance, which becomes weakened if Ca is absent from th surrounding medium. The blastomeres of sea-urchin eggs be come separated in Ca-free sea-water, which is probably du to the absence of this clotting action. Ca will cause the plas magel of amoebae to stiffen. In blood coagulation Ca is neede for the conversion of prothrombin to thrombin, but the plasm Ca has to fall considerably, to levels around 1 mg. per 100 m plasma, before any retardation of clotting occurs.

Nerve is very sensitive to the Ca content of its environment If the Ca level is lowered, the nerve becomes hyperexcitabl and may generate a spontaneous train of impulses. Not onl nerve, but also the sensitivity of ganglia to acetyl choline an in fact the excitability of the whole nervous system may b enhanced by Ca lack. This is seen clinically in tetany associ ated with hypocalcaemia. In the case of nerve, it has bee suggested that 'accommodation', the process whereby nerv resists the action of the exciting stimulus, is depressed.

Ca accelerates the action of several enzymes, but there seem to be an optimal concentration for this to occur, excessiv amounts inhibiting their activity. Blood clotting, which i basically enzymic, is prevented by excess Ca.

Heilbronn considers Ca to play a leading role in the develop ment of bioelectric potentials, due to the existence of a C electrode in the Ca proteinate of the cell cortex. The presen writer feels that the ability of Ca to combine with proteins t form compounds which occur almost everywhere in the body is probably the most significant biological property of thi element, and accounts for its participation in so many physio logical activities.

# Summary

IN the previous chapters the writer has given a survey of the literature and has attempted to weigh up the various findings as fairly as possible. This brief summary is based on his own conclusions from the published observations in the field.

Milk and milk products are the best dietary sources of Ca and pasteurization has little if any effect upon this. Certain vegetables form valuable supplementary sources. It is more important to know the availability of Ca to the body in these foods than the total amounts as there is not necessarily any correlation between the two. Water, even when very hard, is not a reliable source of Ca.

Work with $Ca^{45}$ shows that about half of the dietary Ca is absorbed into the body. A number of factors (e.g. reaction of the diet, fat, protein) have been stated to affect Ca absorption, but from the balance of the evidence it appears that in man the only important single factor is vitamin D. This vitamin also controls the Ca metabolism of bone and possibly renal P excretion. Phytic acid, present in high-extraction flour, has been claimed in England to be significant, in that it depresses Ca absorption in the intestine, but while this has been confirmed experimentally, it is doubtful if it plays such a role in practical dietetics.

In studying the question of Ca requirements, a number of factors have to be taken into account. The so-called endogenous loss, or maintenance requirement, due to processes other than growth, is a very real liability in adult man, and the Ca intake has to meet this to keep the individual in Ca equilibrium. In spite of statements to the contrary, the present writer holds strongly to the view that the previous Ca intake is, within wide limits, the most important controlling factor in

determining the level of Ca intake for equilibrium, owing to the ability of the body to adapt to low dietary levels of Ca. It therefore follows that the Ca requirement is a function of the previous dietary history. With our present dietary habits, certain figures have been arrived at, but they are relevant only to that Ca status. In many studies the significance of the previous Ca intake has not been realized, and as a result the findings for short period balance experiments have no practical meaning and must be discarded.

In growing animals there is no requirement for maintenance and the Ca in the diet is used solely for growth purposes. It should thus be easy to determine, from the daily Ca accretion, what the intake should be. Reliable data as to the composition of the body at different ages are so far lacking, but when such knowledge has been assembled, the problem should be largely solved.

The utilization of Ca in the body is high in many animals, but it is much lower in well-nourished children and adults, between 20 and 30 per cent. of that ingested. It may be much higher in individuals on a previously lower Ca intake. In old people an osteoporosis often develops, due to a combination of endocrine imbalance, inactivity and possibly Ca deficiency.

Pregnancy does not impose a strain on the Ca metabolism of the mother as the Ca content of the foetus at term is not high. Lactation, on the other hand, may lead to quite severe depletion unless extra Ca is given in the diet. Even so, the mother may find the Ca of her bones more available for her milk than that of her food.

The Ca level of the blood is remarkably constant; Ca is present in two fractions, ionized and non-diffusible, the latter being combined with protein. In man, virtually all the Ca is in the plasma. A large number of factors, both dietary and endocrine, control the blood Ca level, the most important endocrine influence coming from the parathyroids. Two views have been adumbrated, one that the gland acts on Ca mobilization from bone, and the other that it controls renal phosphate excretion. The balance of evidence supports the former view,

though recent work suggests that there are two factors, hypercalcaemic and phosphaturic, in parathyroid extracts.

The histology and biochemistry of bone are becoming increasingly interconnected, especially owing to the use of the rachitic epiphysis which will calcify under appropriate conditions *in vitro*. Using this technique, it has been shown that the glycolytic cycle may under certain conditions govern cartilage calcification, though it is possible for cartilage to calcify with all enzymes inactivated. Quite frankly, the present writer cannot see the solution to this discrepancy, but advances are so rapid in this field that the problem will doubtless soon be solved. Robison's alkaline phosphatase seems to be solely concerned with matrix formation, and not with calcification. There is strong and increasing evidence that mucopolysaccharides are intimately related to calcification, by acting as a template for crystallization.

The evidence is now unanimous that bone salt has an apatite structure, the differences in composition (e.g. Ca : P ratio) in bone being accounted for by surface adsorption. The total surface area of the bone crystals in an adult man has been calculated to be over 100 acres, which is in equilibrium with the blood-plasma, and accounts in part for the constancy of the blood Ca level. The process of mineralization of bone is a crystallization and not a precipitation from supersaturated solutions, and no solubility product has yet been demonstrated to be exceeded. However, calcification is in some ways correlated with the product $[Ca^{++}] \times [HPO_4^{--}]$ which seems to determine the upper limit for Ca and P ions in solution. Bone formation is governed by almost all the endocrine glands and several of the vitamins. The most extreme change caused by dietary imbalance is rickets, due in man to lack of vitamin D, when the calcification sequences slow and finally cease.

Teeth differ from bones in having only a one-way process of calcification and no resorption can take place. The fully erupted tooth is metabolically very inactive compared with bone, but the unerupted tooth and the continually erupting rodent incisor have a high Ca metabolism. The mineral in both

enamel and dentin has an apatite structure. It seems likely that mucopolysaccharides are implicated in calcification as they are in bone. Several vitamins and endocrine glands control the formation of dental tissues.

The bulk of Ca lost from the body appears in the faeces, but most of this is unabsorbed dietary Ca. There seems no doubt that active Ca excretion from the body occurs via the gut in man, but it may be a reserve mechanism not usually employed to any extent. Active Ca excretion by this route also occurs in animals. Recent work suggests that the kidney tubule re-absorbs ionic Ca very efficiently, and the Ca lost in the urine is in the form of unionized complexes which the tubule cannot absorb. This is the most convincing explanation up to now of the constant Ca loss in the urine. Sweat forms a vehicle for Ca loss which though relatively small is not insignificant.

In conclusion it can be stated that there is hardly any physiological action which does not involve the intervention of Ca.

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(Unless otherwise stated, phosphatase refers to alkaline phosphatase)